OLIGODEOXYNUCLEOTIDE Xol26

MstII

5'-CCCGGGAAGCTTCCTTAGGCTTAAAGAAAGTGGTGCTGGGCAAAAAAGGG-3'

prepro-HSA ← → V1 domain of CD4 receptor

OLIGODEOXYNUCLEOTIDE Xol27

SmaI HindIII

5'-CCCGGGAAGCTTTTAGAAAGCTAGCACCACGATGTCTAT-3'

→ V2 domain of CD4 receptor

stop codon

Figure 1

10073418.022102

```
 MstII
CCTTAGGCTTAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTTCCCAGAAGA
01        11        21        31        41        51        61        71

AGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAGATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAG
76        86        96        106       116       126       136       146

GTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCA
151       161       171       181       191       201       211       221

AGAATCTTAAGATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAG
226       236       246       256       266       276       286       296

TGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGGAGAGCCCCC
301       311       321       331       341       351       361       371

CTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATACAGGGGGGGAAGACCCTCTCCGTGT
376       386       396       406       416       426       436       446

CTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAA
451       461       471       481       491       501       511       521

                             HindIII Smal
TAGACATCGTGGTGCTAGCTTTCTAAAAGCTTCCCGGG
526       536       546       556
```

Figure 2

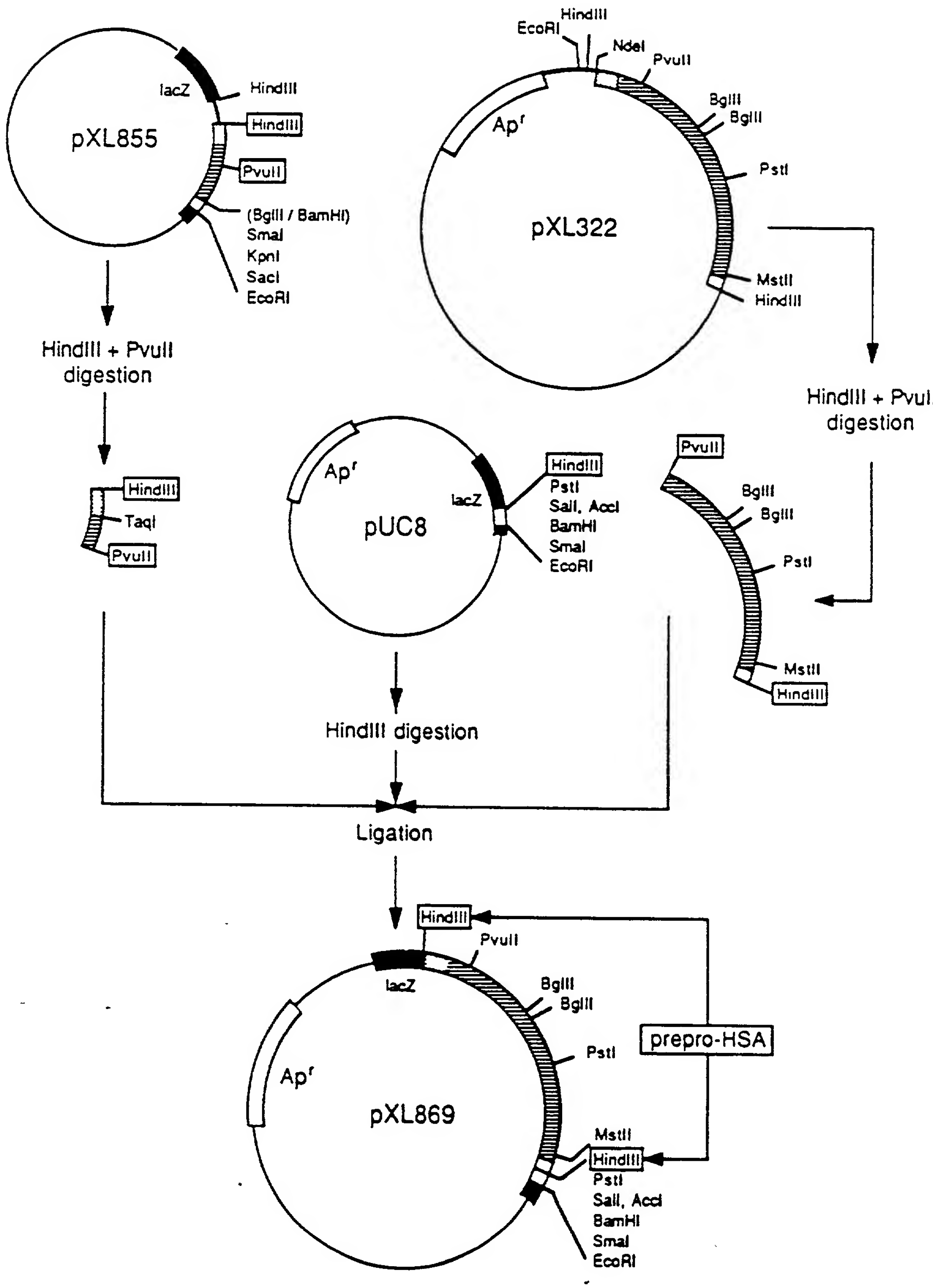

pXL855
lacZ
HindIII
HindIII
PvuII
(BglII / BamHI)
SmaI
KpnI
SacI
EcoRI
HindIII + PvuII digestion
HindIII
TaqI
PvuII
pXL322
EcoRI
HindIII
NdeI
PvuII
Ap^r
BglII
BglII
PstI
MstII
HindIII
HindIII + PvuII digestion
PvuII
BglII
BglII
PstI
MstII
HindIII
pUC8
Ap^r
lacZ
HindIII
PstI
SalI, AccI
BamHI
SmaI
EcoRI
HindIII digestion
Ligation
pXL869
HindIII
PvuII
lacZ
BglII
BglII
PstI
prepro-HSA
Ap^r
MstII
HindIII
PstI
SalI, AccI
BamHI
SmaI
EcoRI

Figure 3

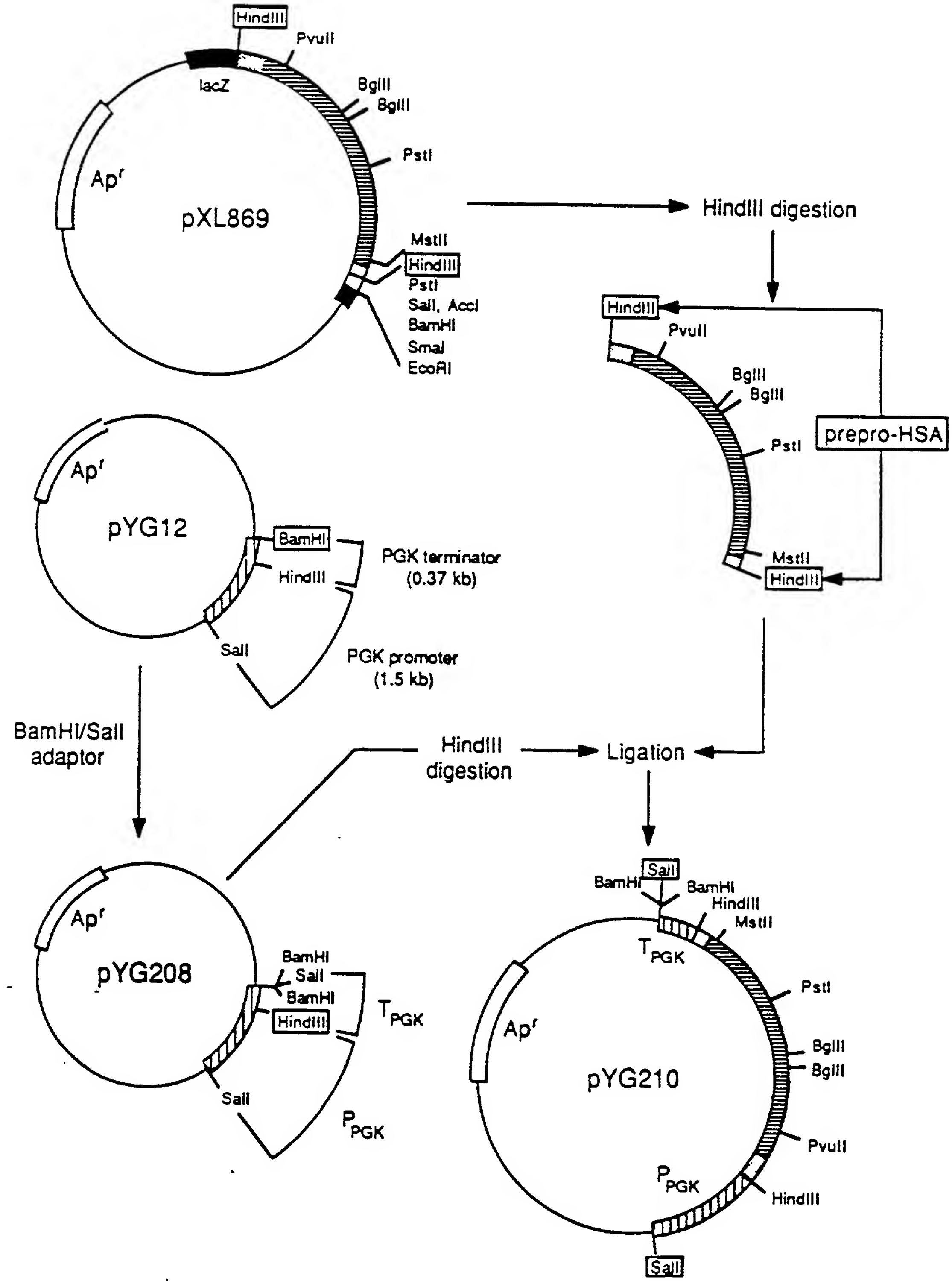


Figure 4

20070210 "0274420

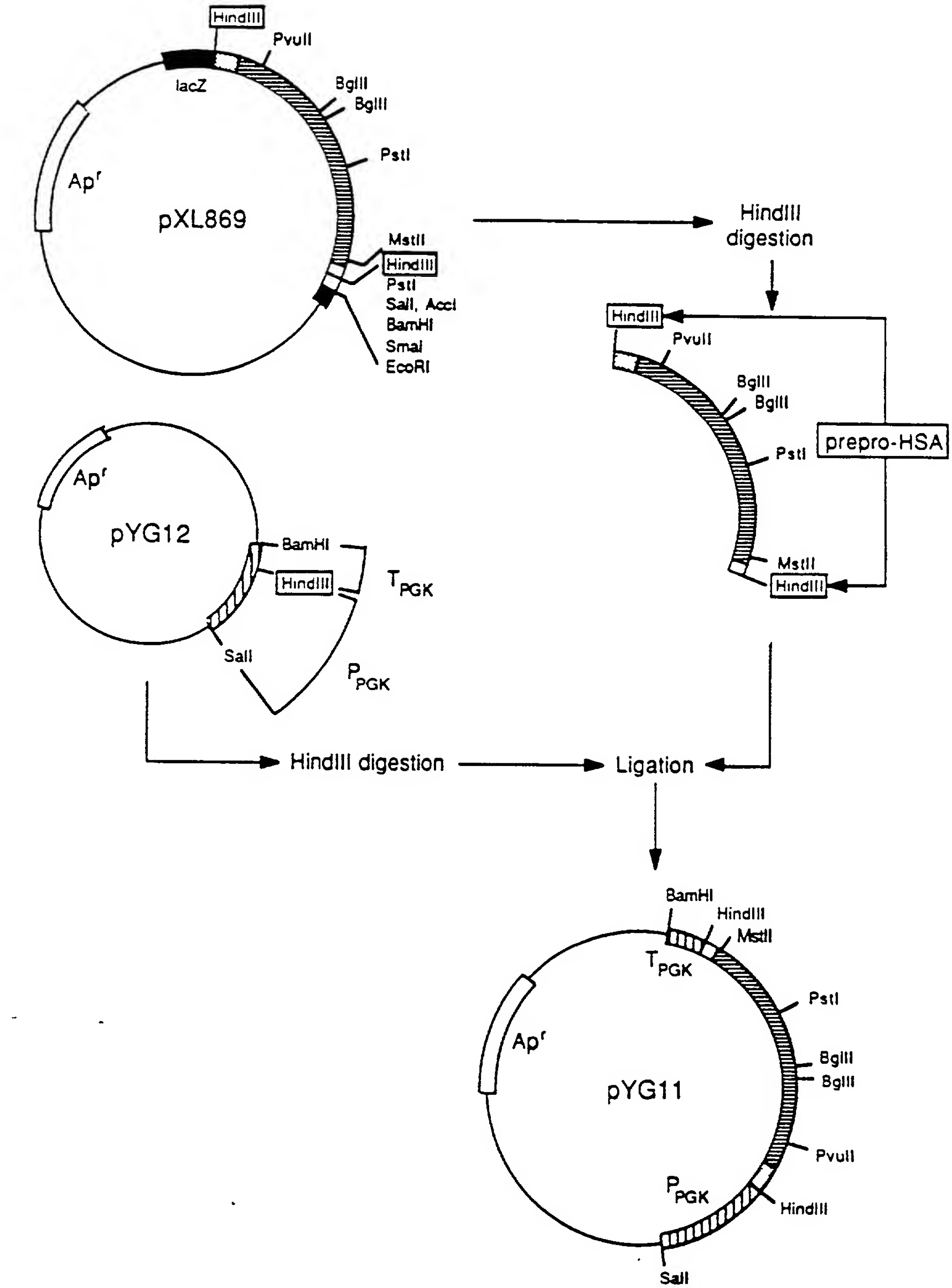
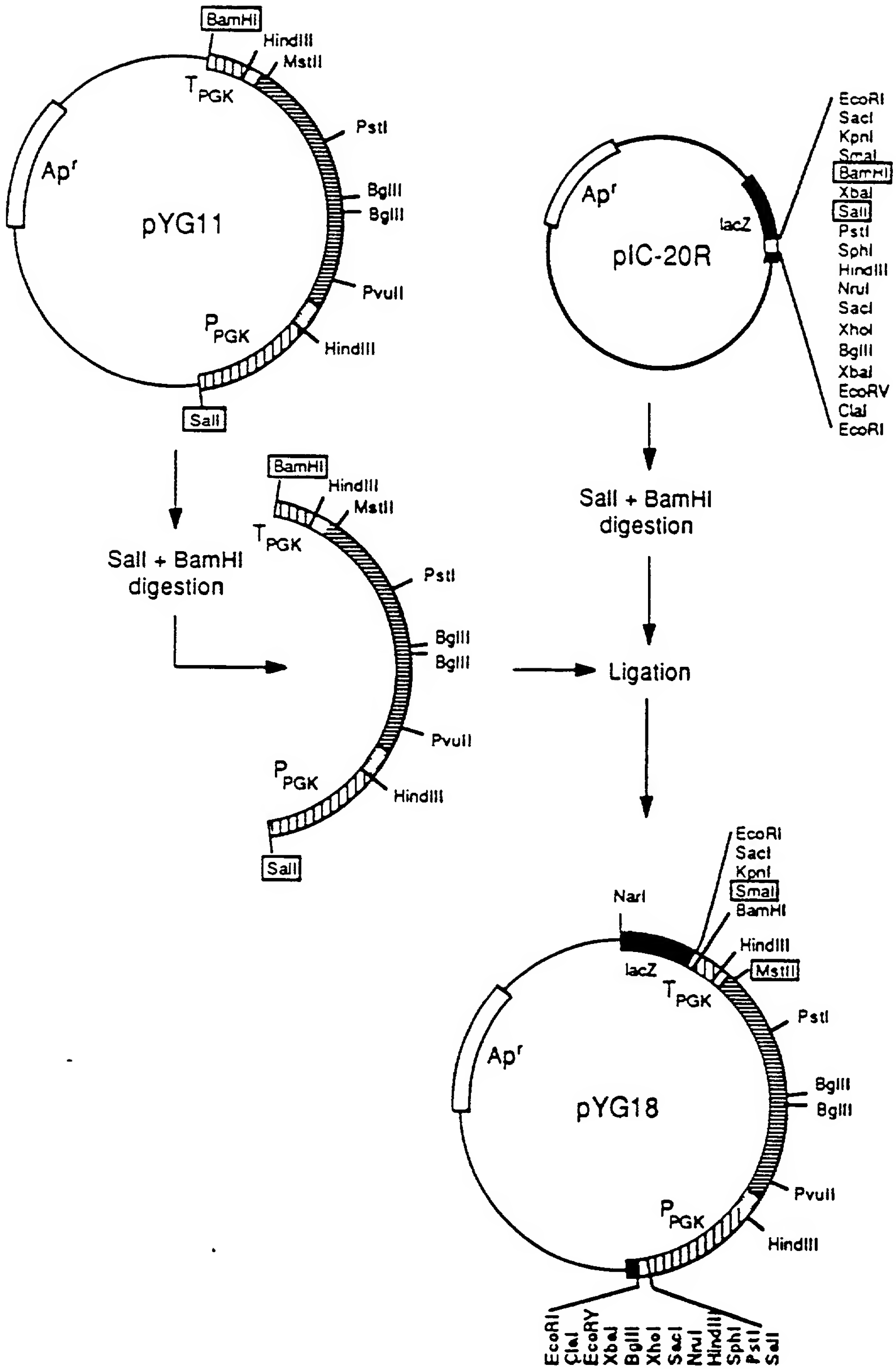


Figure 5

20290 " 07163007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HindIII
MstII
T_{PGK}
PstI
BglII
BglII
Ap^r
pYG11
PvuII
P_{PGK}
HindIII
SalI
pIC-20R
lacZ
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
NruI
SacI
XhoI
BglII
XbaI
EcoRV
ClaI
EcoRI
SalI + BamHI digestion
SalI + BamHI digestion
Ligation
NarI
pYG18
EcoRI
ClaI
EcoRV
XbaI
BglII
XhoI
SacI
NruI
HindIII
SphI
PstI
SalI

Figure 6

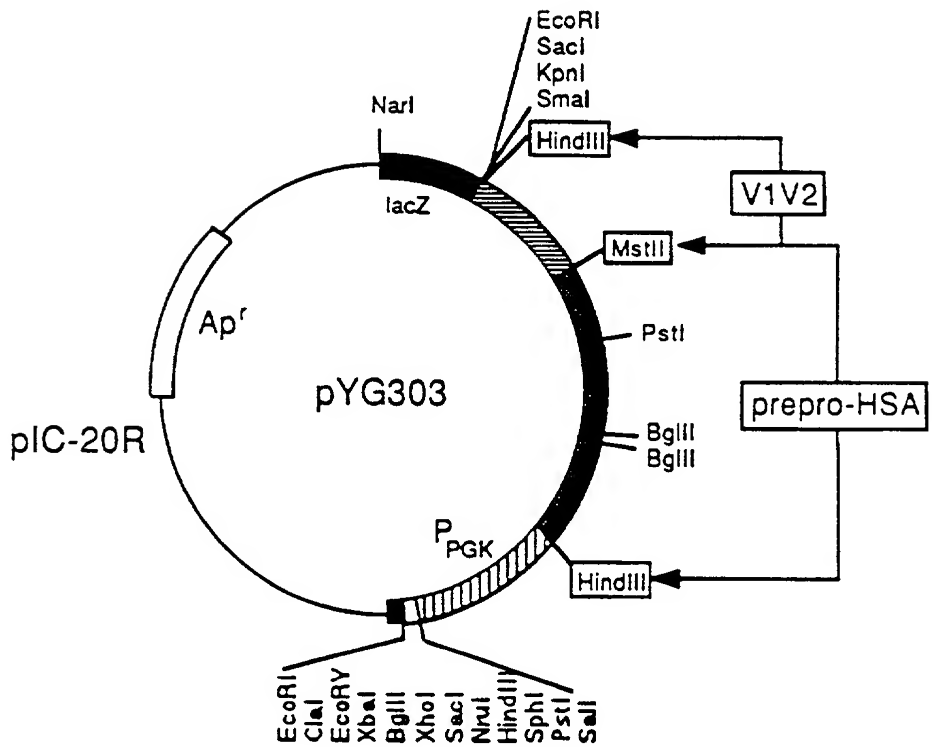

EcoRI
SacI
KpnI
SmaI
NarI
HindIII
V1V2
lacZ
MstII
PstI
Ap^r
pYG303
prepro-HSA
BglII
BglII
pIC-20R
P PGK
HindIII
EcoRI
ClaI
EcoRV
XbaI
BglII
XhoI
SacI
NruI
HindIII
SphI
PstI
SalI

Figure 7

```
         MetLysTrpValThrPheIleSerLeuLeuPheLeuPheSerSerAlaTyrSerArgGlyValPheArg
AAGCTTATGAAGTGGGTAACCTTTATTTCCCTTCTTTTTCTCTTTAGCTCGGCTTATTCCAGGGGTGTGTTTCGT
1         11        21        31        41        51        61        71

ArgAspAlaHisLysSerGluValAlaHisArgPheLysAspLeuGlyGluGluAsnPheLysAlaLeuValLeu
CGAGATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAGCCTTGGTGTTG
76        86        96        106       116       126       136       146

IleAlaPheAlaGlnTyrLeuGlnGlnCysProPheGluAspHisValLysLeuValAsnGluValThrGluPhe
ATTGCCTTTGCTCAGTATCTTCAGCAGTGTCCATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAATTT
151       161       171       181       191       201       211       221

AlaLysThrCysValAlaAspGluSerAlaGluAsnCysAspLysSerLeuHisThrLeuPheGlyAspLysLeu
GCAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTA
226       236       246       256       266       276       286       296

CysThrValAlaThrLeuArgGluThrTyrGlyGluMetAlaAspCysCysAlaLysGlnGluProGluArgAsn
TGCACAGTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGTGCAAAACAAGAACCTGAGAGAAAT
301       311       321       331       341       351       361       371

GluCysPheLeuGlnHisLysAspAspAsnProAsnLeuProArgLeuValArgProGluValAspValMetCys
GAATGCTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCCGATTGGTGAGACCAGAGGTTGATGTGATGTGC
376       386       396       406       416       426       436       446

ThrAlaPheHisAspAsnGluGluThrPheLeuLysLysTyrLeuTyrGluIleAlaArgArgHisProTyrPhe
ACTGCTTTTCATGACAATGAAGAGACATTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTACTTT
451       461       471       481       491       501       511       521

TyrAlaProGluLeuLeuPhePheAlaLysArgTyrLysAlaAlaPheThrGluCysCysGlnAlaAlaAspLys
TATGCCCCGGAACTCCTTTTCTTTGCTAAAAGGTATAAAGCTGCTTTTACAGAATGTTGCCAAGCTGCTGATAAA
526       536       546       556       566       576       586       596

AlaAlaCysLeuLeuProLysLeuAspGluLeuArgAspGluGlyLysAlaSerSerAlaLysGlnArgLeuLys
GCTGCCTGCCTGTTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTCTGCCAAACAGAGACTCAAG
601       611       621       631       641       651       661       671

CysAlaSerLeuGlnLysPheGlyGluArgAlaPheLysAlaTrpAlaValAlaArgLeuSerGlnArgPhePro
TGTGCCAGTCTCCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGATTTCCC
676       686       696       706       716       726       736       746

LysAlaGluPheAlaGluValSerLysLeuValThrAspLeuThrLysValHisThrGluCysCysHisGlyAsp
AAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGACAGATCTTACCAAAGTCCACACGGAATGCTGCCATGGAGAT
751       761       771       781       791       801       811       821

LeuLeuGluCysAlaAspAspArgAlaAspLeuAlaLysTyrIleCysGluAsnGlnAspSerIleSerSerLys
CTGCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAAATCAAGATTCGATCTCCAGTAAA
826       836       846       856       866       876       886       896

LeuLysGluCysCysGluLysProLeuLeuGluLysSerHisCysIleAlaGluValGluAsnAspGluMetPro
CTGAAGGAATGCTGTGAAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAAAATGATGAGATGCCT
901       911       921       931       941       951       961       971

AlaAspLeuProSerLeuAlaAlaAspPheValGluSerLysAspValCysLysAsnTyrAlaGluAlaLysAsp
GCTGACTTGCCTTCATTAGCTGCTGATTTTGTTGAAAGTAAGGATGTTTGCAAAAACTATGCTGAGGCAAAGGAT
976       986       996       1006      1016      1026      1036      1046
```

Figure 8A

```
    ValPheLeuGlyMetPheLeuTyrGluTyrAlaArgArgHisProAspTyrSerValValLeuLeuLeuArgLeu
    GTCTTCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATTACTCTGTCGTACTGCTGCTGAGACTT
1051      1061      1071      1081      1091      1101      1111      1121

    AlaLysThrTyrGluThrThrLeuGluLysCysCysAlaAlaAlaAspProHisGluCysTyrAlaLysValPhe
    GCCAAGACATATGAAACCACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTATGCCAAAGTGTTC
1126      1136      1146      1156      1166      1176      1186      1196

    AspGluPheLysProLeuValGluGluProGlnAsnLeuIleLysGlnAsnCysGluLeuPheGluGlnLeuGly
    GATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTGAGCTTTTTGAGCAGCTTGGA
1201      1211      1221      1231      1241      1251      1261      1271

    GluTyrLysPheGlnAsnAlaLeuLeuValArgTyrThrLysLysValProGlnValSerThrProThrLeuVal
    GAGTACAAATTCCAGAATGCGCTATTAGTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCCAACTCTTGTA
1276      1286      1296      1306      1316      1326      1336      1346

    GluValSerArgAsnLeuGlyLysValGlySerLysCysCysLysHisProGluAlaLysArgMetProCysAla
    GAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCA
1351      1361      1371      1381      1391      1401      1411      1421

    GluAspTyrLeuSerValValLeuAsnGlnLeuCysValLeuHisGluLysThrProValSerAspArgValThr
    GAAGACTATCTATCCGTGGTCCTGAACCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAGTCACC
1426      1436      1446      1456      1466      1476      1486      1496

    LysCysCysThrGluSerLeuValAsnArgArgProCysPheSerAlaLeuGluValAspGluThrTyrValPro
    AAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAAGTCGATGAAACATACGTTCCC
1501      1511      1521      1531      1541      1551      1561      1571

    LysGluPheAsnAlaGluThrPheThrPheHisAlaAspIleCysThrLeuSerGluLysGluArgGlnIleLys
    AAAGAGTTTAATGCTGAAACATTCACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAGACAAATCAAG
1576      1586      1596      1606      1616      1626      1636      1646

    LysGlnThrAlaLeuValGluLeuValLysHisLysProLysAlaThrLysGluGlnLeuLysAlaValMetAsp
    AAACAAACTGCACTTGTTGAGCTTGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTATGGAT
1651      1661      1671      1681      1691      1701      1711      1721

    AspPheAlaAlaPheValGluLysCysCysLysAlaAspAspLysGluThrCysPheAlaGluGluGlyLysLys
    GATTTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAA
1726      1736      1746      1756      1766      1776      1786      1796

    LeuValAlaAlaSerGlnAlaAlaLeuGlyLeuLysLysValValLeuGlyLysLysGlyAspThrValGluLeu
    CTTGTTGCTGCAAGTCAAGCTGCCTTAGGCTTAAAGAAAGTGGTGCTGGGCAAAAAGGGGATACAGTGGAACTG
1801      1811      1821      1831      1841      1851      1861      1871

    ThrCysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnIleLysIleLeuGlyAsn
    ACCTGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAGATTCTGGGAAAT
1876      1886      1896      1906      1916      1926      1936      1946

    GlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgAlaAspSerArgArgSerLeuTrpAspGln
    CAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAAGAAGCCTTTGGGACCAA
1951      1961      1971      1981      1991      2001      2011      2021

    GlyAsnPheProLeuIleIleLysAsnLeuLysIleGluAspSerAspThrTyrIleCysGluValGluAspGln
    GGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGGACCAG
2026      2036      2046      2056      2066      2076      2086      2096

    LysGluGluValGlnLeuLeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeu
    AAGGAGGAGGTGCAATTGCTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTG
2101      2111      2121      2131      2141      2151      2161      2171
```

Figure 8B

```
   ThrLeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGlyLysAsnIleGln
   ACCCTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATACAG
2176      2186      2196      2206      2216      2226      2236      2246

   GlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGlyThrTrpThrCysThrValLeuGlnAsn
   GGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACATGCACTGTCTTGCAGAAC
2251      2261      2271      2281      2291      2301      2311      2321

   GlnLysLysValGluPheLysIleAspIleValValLeuAlaPhe***
   CAGAAGAAGGTGGAGTTCAAAATAGACATCGTGGTGCTAGCTTTCTAAAAGCTT
2326      2336      2346      2356      2366      2376
```

10073118.021202

Figure 8C

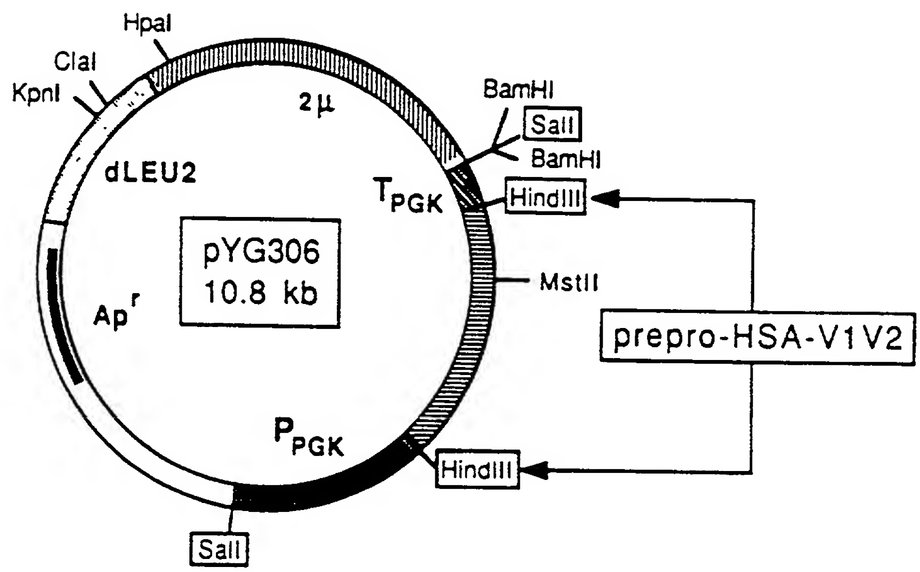
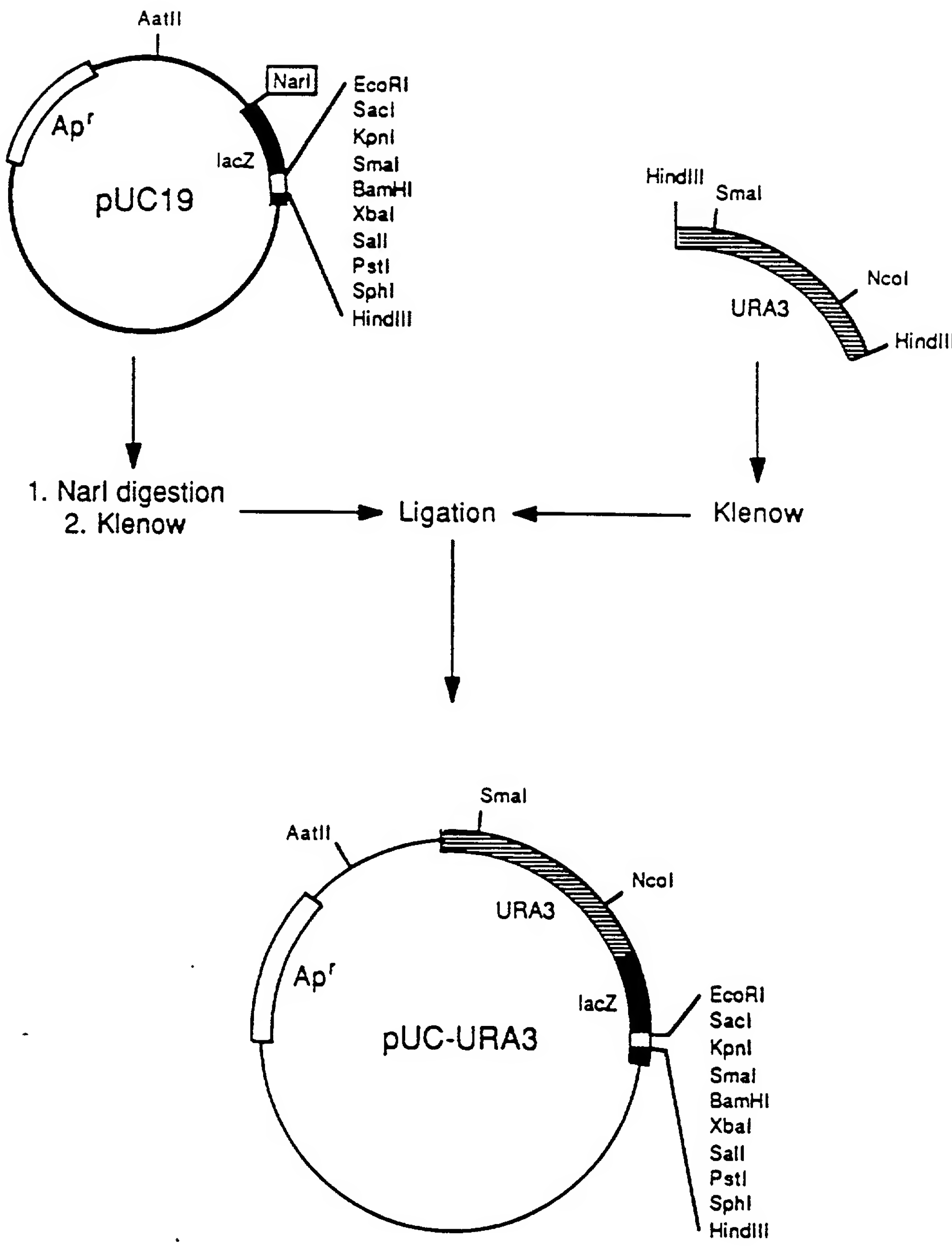

HpaI
ClaI
KpnI
2µ
dLEU2
BamHI
SalI
BamHI
T_{PGK}
HindIII
pYG306
10.8 kb
MstII
prepro-HSA-V1V2
Ap^r
P_{PGK}
HindIII
SalI

Figure 9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NarI
Ap^r
pUC19
lacZ
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
HindIII
SmaI
NcoI
URA3
HindIII
1. NarI digestion
2. Klenow
Ligation
Klenow
SmaI
AatII
NcoI
URA3
Ap^r
lacZ
pUC-URA3
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII

Figure 10

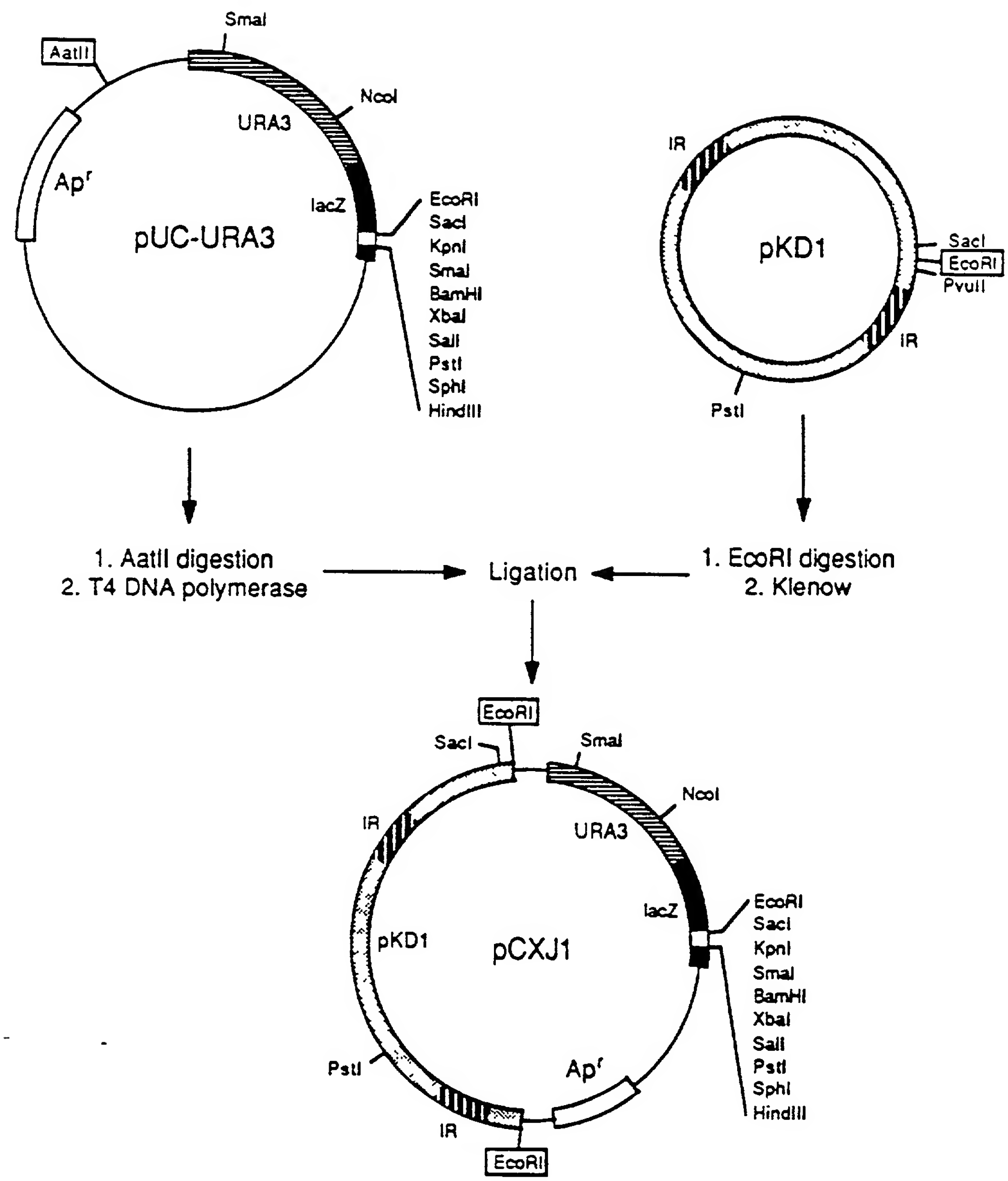


Figure 11

20210203.131807

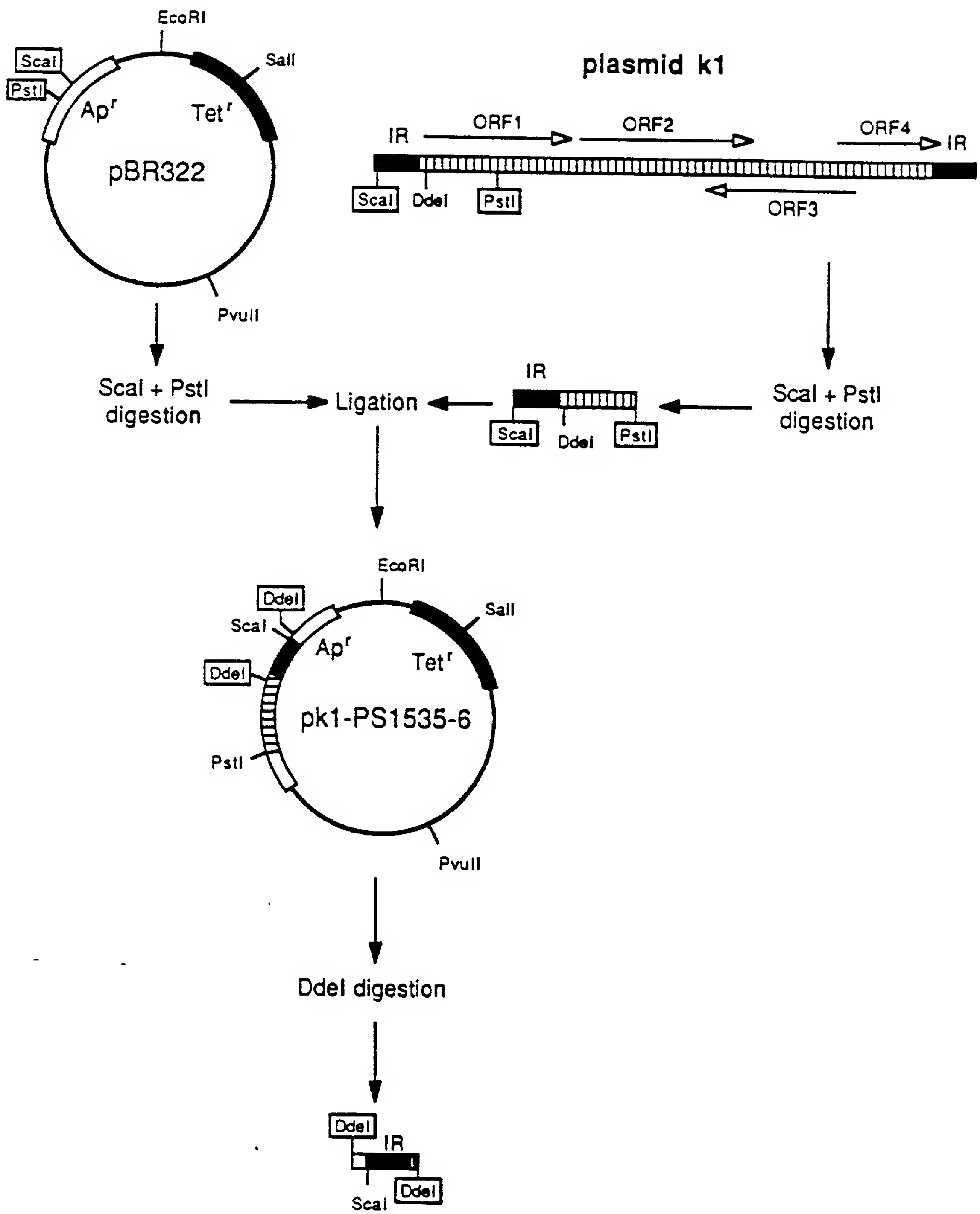
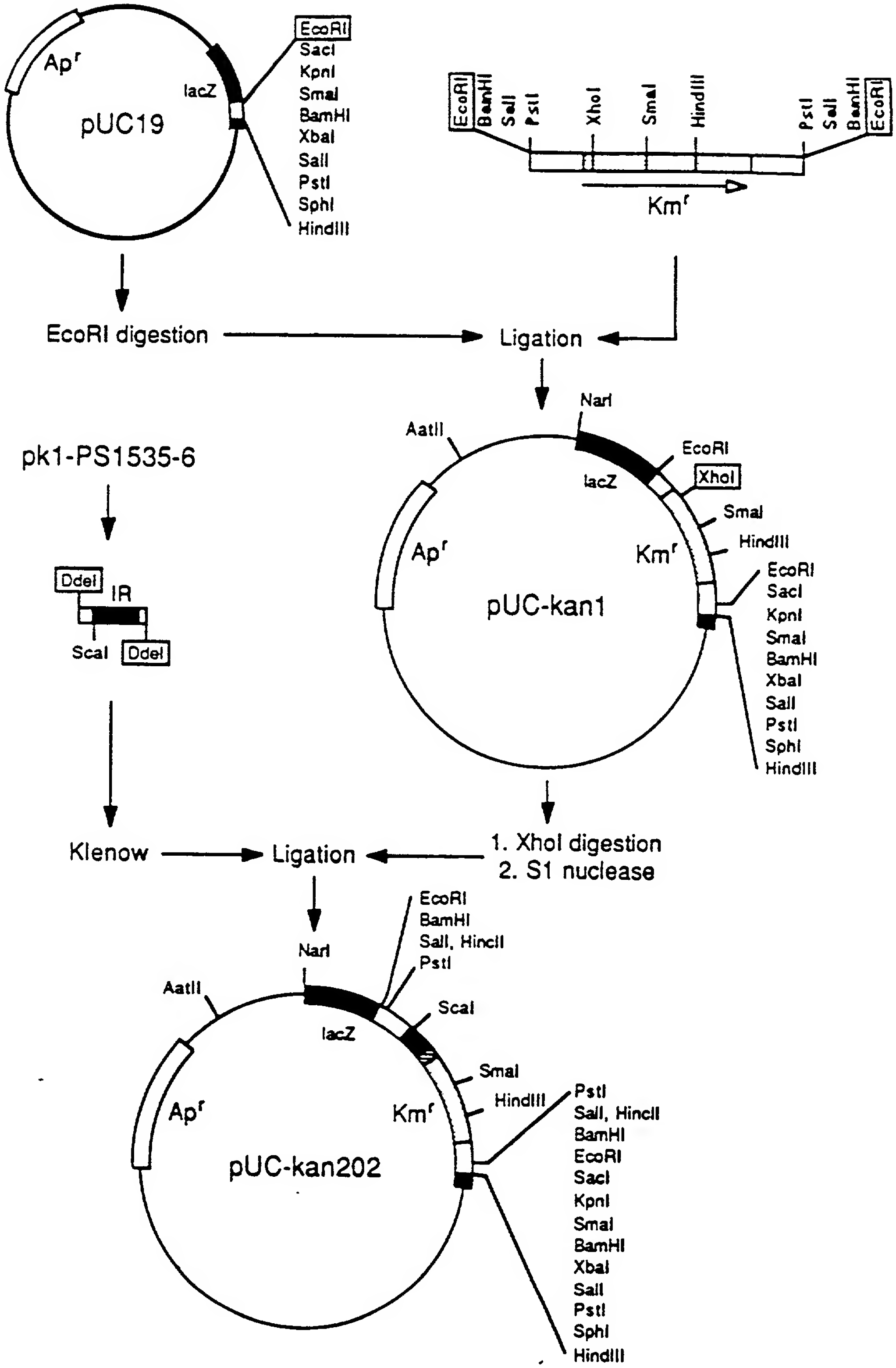
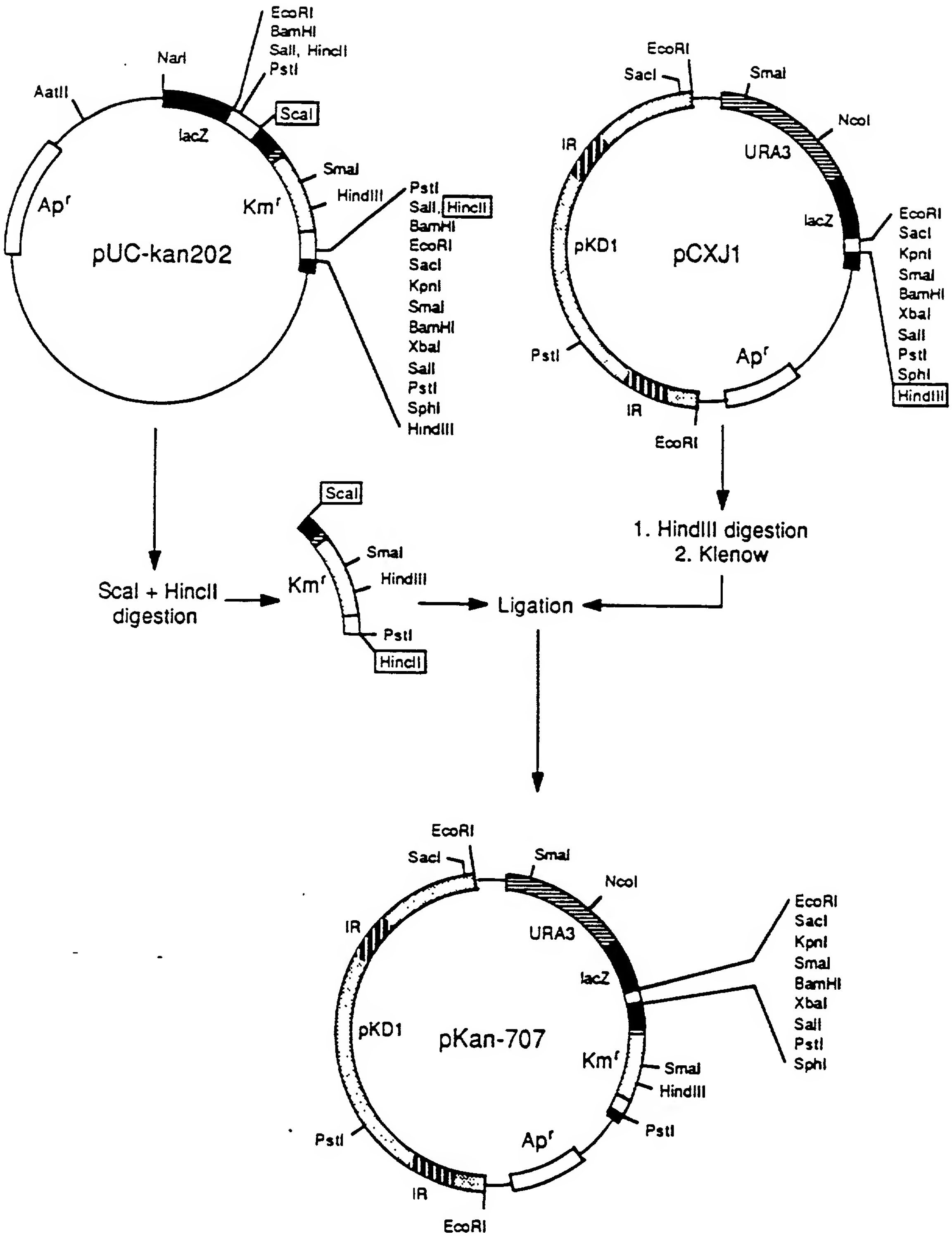

EcoRI
ScaI
PstI
SalI
Apr
Tetr
pBR322
PvuII
plasmid k1
IR
ORF1
ORF2
ORF4
IR
ScaI
DdeI
PstI
ORF3
ScaI + PstI
digestion
Ligation
IR
ScaI
DdeI
PstI
ScaI + PstI
digestion
EcoRI
DdeI
ScaI
SalI
Apr
Tetr
DdeI
pk1-PS1535-6
PstI
PvuII
DdeI digestion
DdeI
IR
ScaI
DdeI

Figure 12



pUC19
Ap^r
lacZ
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
EcoRI BamHI SalI PstI XhoI SmaI HindIII PstI SalI BamHI EcoRI
Km^r
EcoRI digestion
Ligation
pk1-PS1535-6
DdeI
IR
ScaI
DdeI
pUC-kan1
NarI
AatII
lacZ
EcoRI
XhoI
SmaI
HindIII
Ap^r
Km^r
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
Klenow
Ligation
1. XhoI digestion
2. S1 nuclease
pUC-kan202
NarI
AatII
lacZ
EcoRI
BamHI
SalI, HincII
PstI
ScaI
SmaI
HindIII
Ap^r
Km^r
PstI
SalI, HincII
BamHI
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII

Figure 13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BamHI
SalI, HincII
PstI
NarI
AatII
ScaI
lacZ
SmaI
HindIII
Ap^r
Km^r
pUC-kan202
PstI
SalI, HincII
BamHI
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
EcoRI
SacI
SmaI
NcoI
IR
URA3
pKD1
pCXJ1
lacZ
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
PstI
Ap^r
IR
EcoRI
ScaI
SmaI
HindIII
Km^r
PstI
HincII
ScaI + HincII
digestion
1. HindIII digestion
2. Klenow
Ligation
EcoRI
SacI
SmaI
NcoI
IR
URA3
lacZ
pKD1
pKan-707
Km^r
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
SmaI
HindIII
PstI
PstI
Ap^r
IR
EcoRI

Figure 14

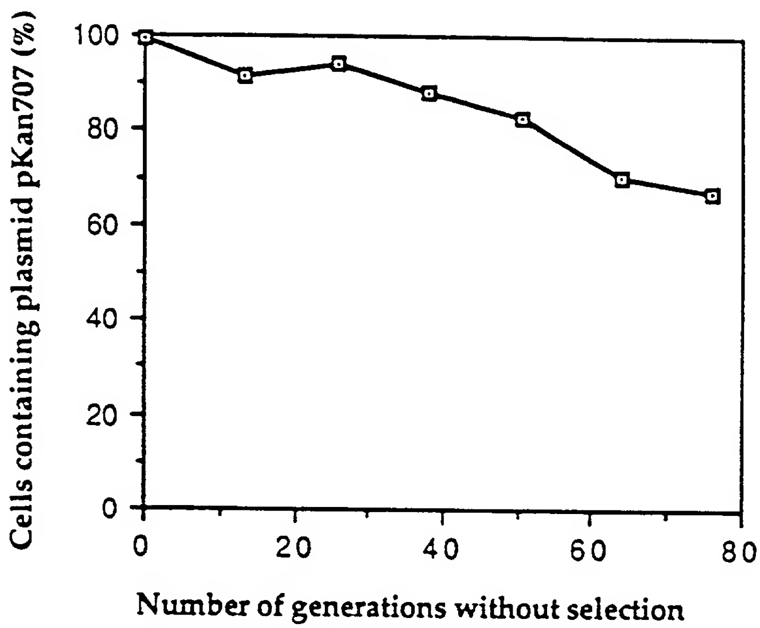

Cells containing plasmid pKan707 (%)
100
80
60
40
20
0
0
20
40
60
80
Number of generations without selection

Figure 15

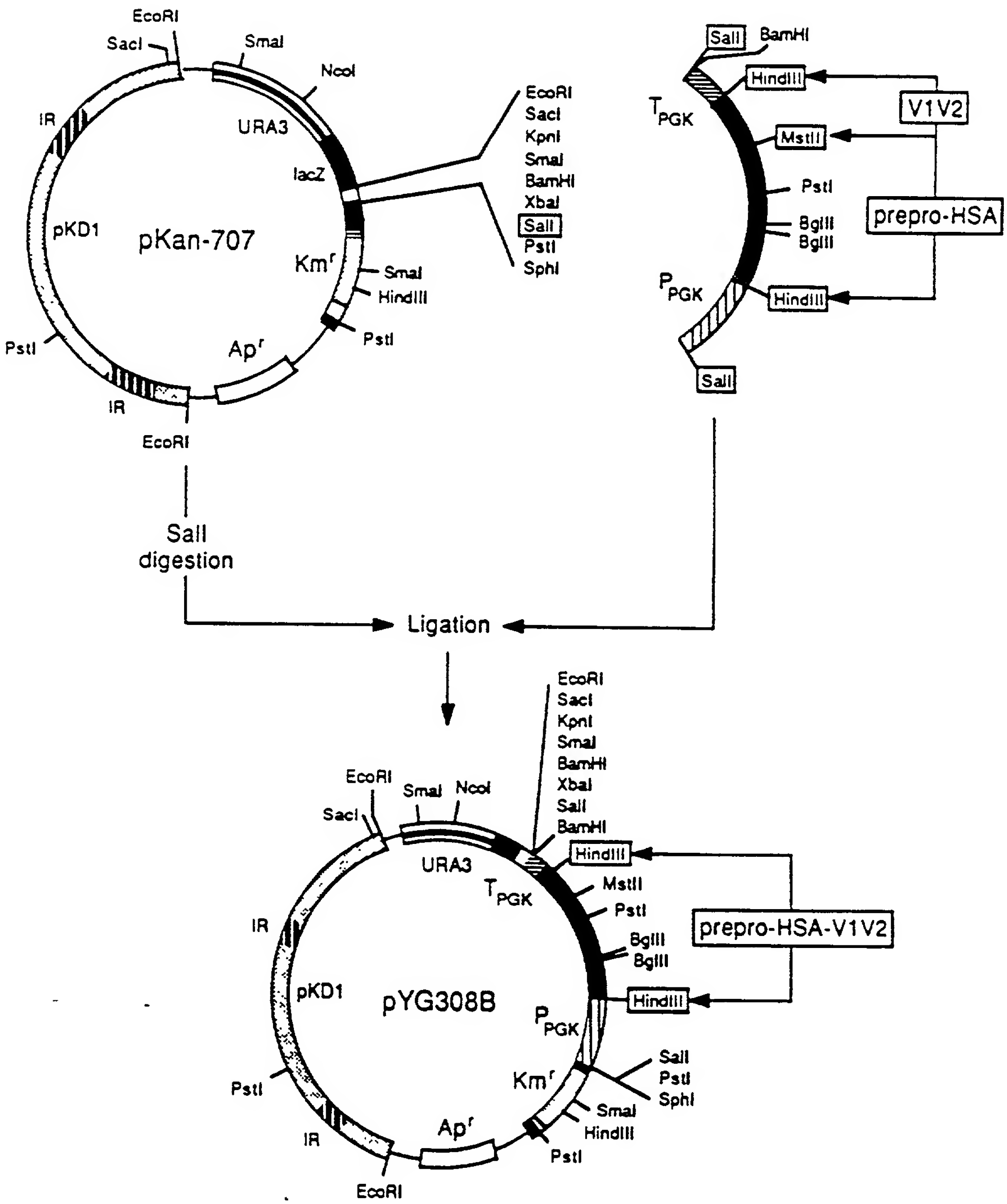

EcoRI
SacI
SmaI
NcoI
IR
URA3
lacZ
pKD1
pKan-707
Km^r
SmaI
HindIII
PstI
Ap^r
PstI
IR
EcoRI
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
SalI
BamHI
HindIII
V1V2
T PGK
MstII
PstI
BglII
BglII
prepro-HSA
P PGK
HindIII
SalI
SalI
digestion
Ligation
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
BamHI
HindIII
EcoRI
SmaI
NcoI
SacI
URA3
T PGK
MstII
PstI
BglII
BglII
prepro-HSA-V1V2
IR
pKD1
pYG308B
P PGK
HindIII
SalI
PstI
SphI
Km^r
SmaI
HindIII
PstI
Ap^r
IR
EcoRI

Figure 16

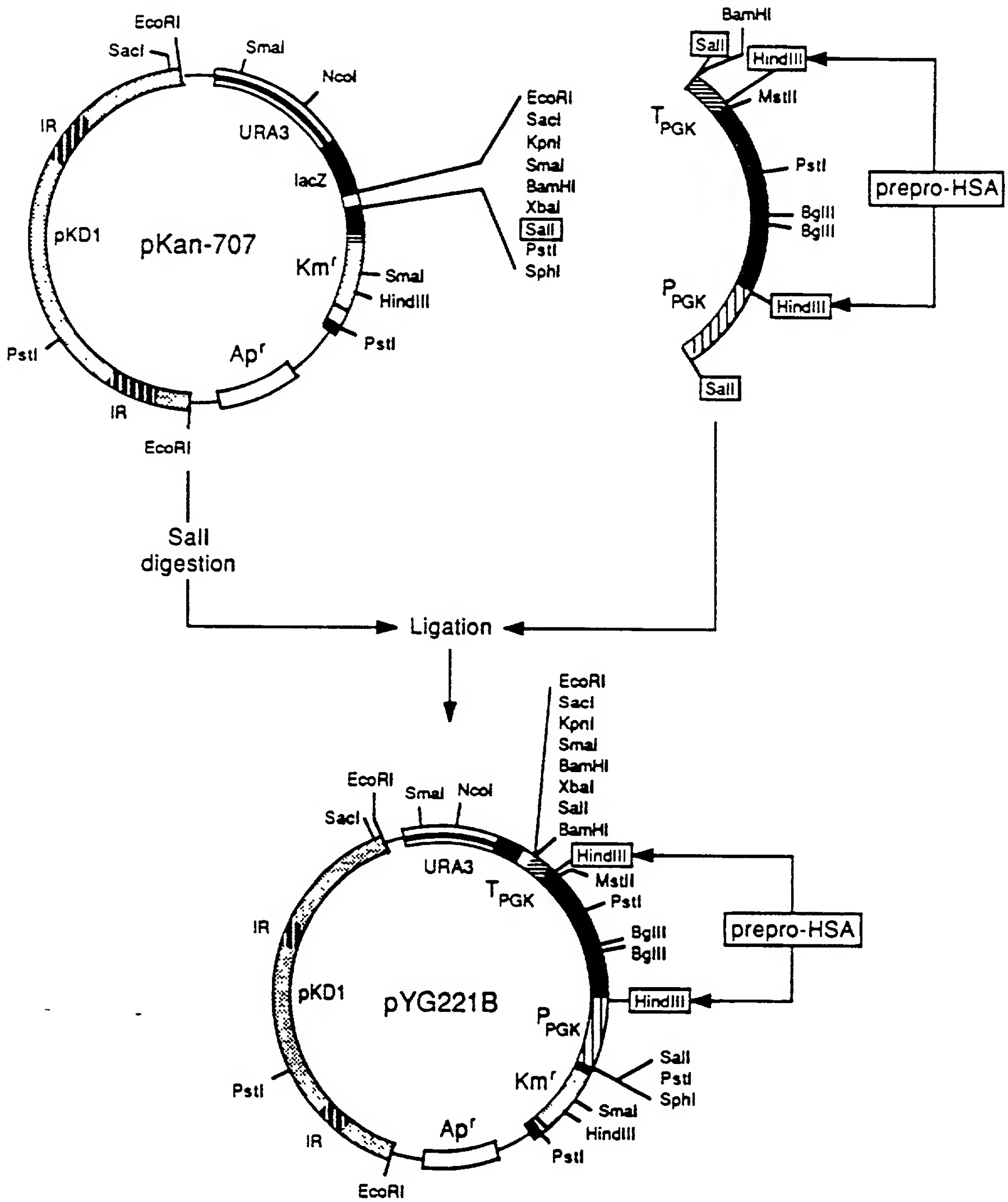

EcoRI
SacI
SmaI
NcoI
IR
URA3
lacZ
pKD1
pKan-707
Km^r
SmaI
HindIII
PstI
Ap^r
PstI
IR
EcoRI
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
BamHI
SalI
HindIII
MstII
T_{PGK}
PstI
prepro-HSA
BglII
BglII
P_{PGK}
HindIII
SalI
SalI digestion
Ligation
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
BamHI
EcoRI
SmaI
NcoI
SacI
HindIII
URA3
MstII
T_{PGK}
PstI
IR
BglII
BglII
prepro-HSA
pKD1
pYG221B
HindIII
P_{PGK}
SalI
PstI
SphI
Km^r
PstI
SmaI
HindIII
Ap^r
IR
PstI
EcoRI

Figure 17

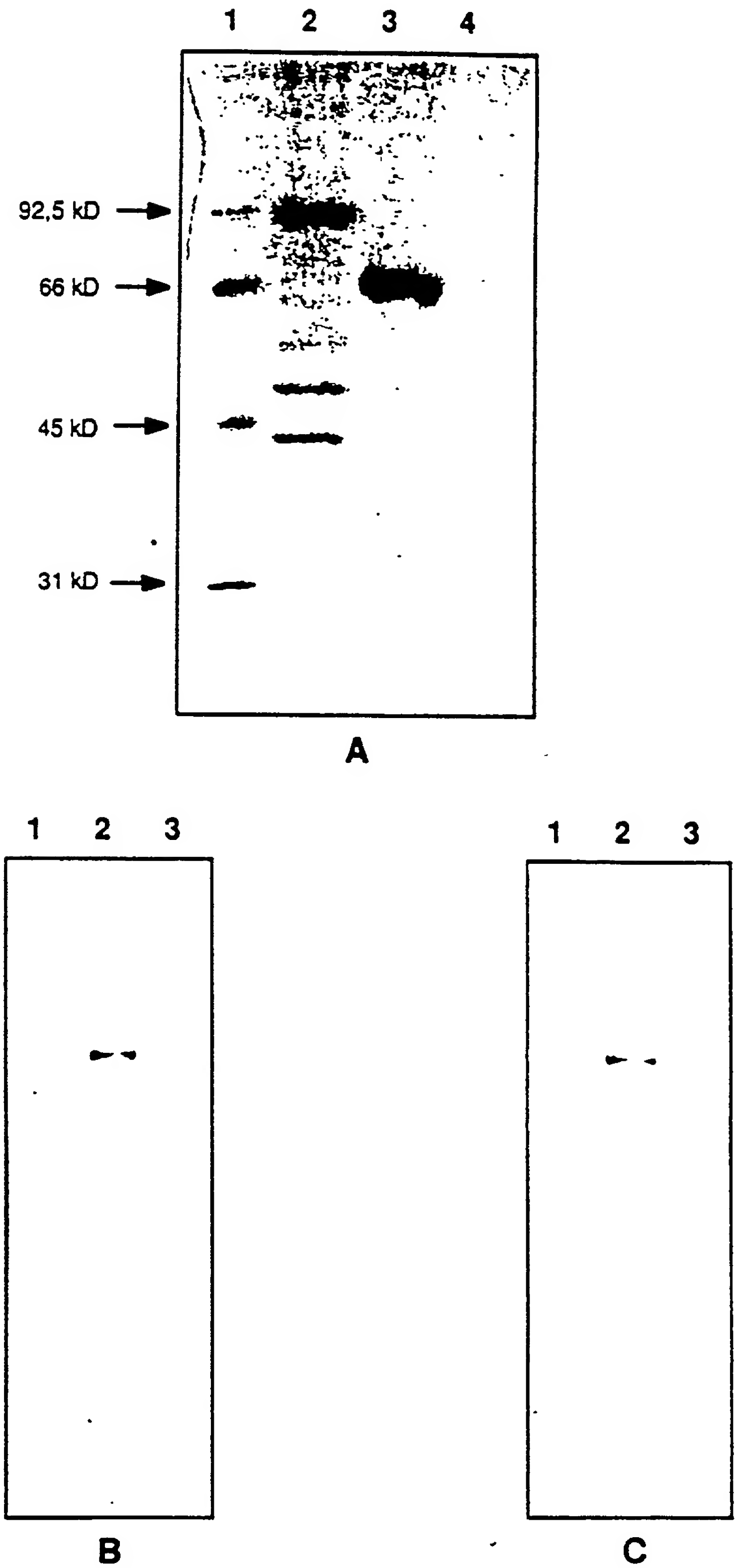


Figure 18

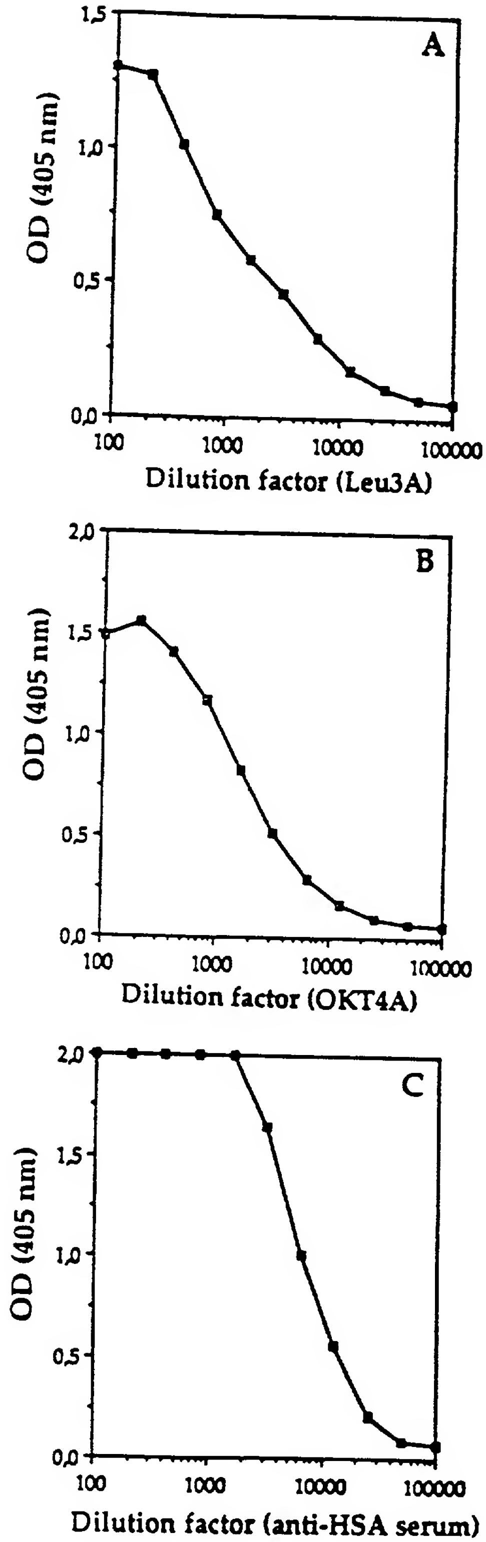


Figure 19

20212022.031501

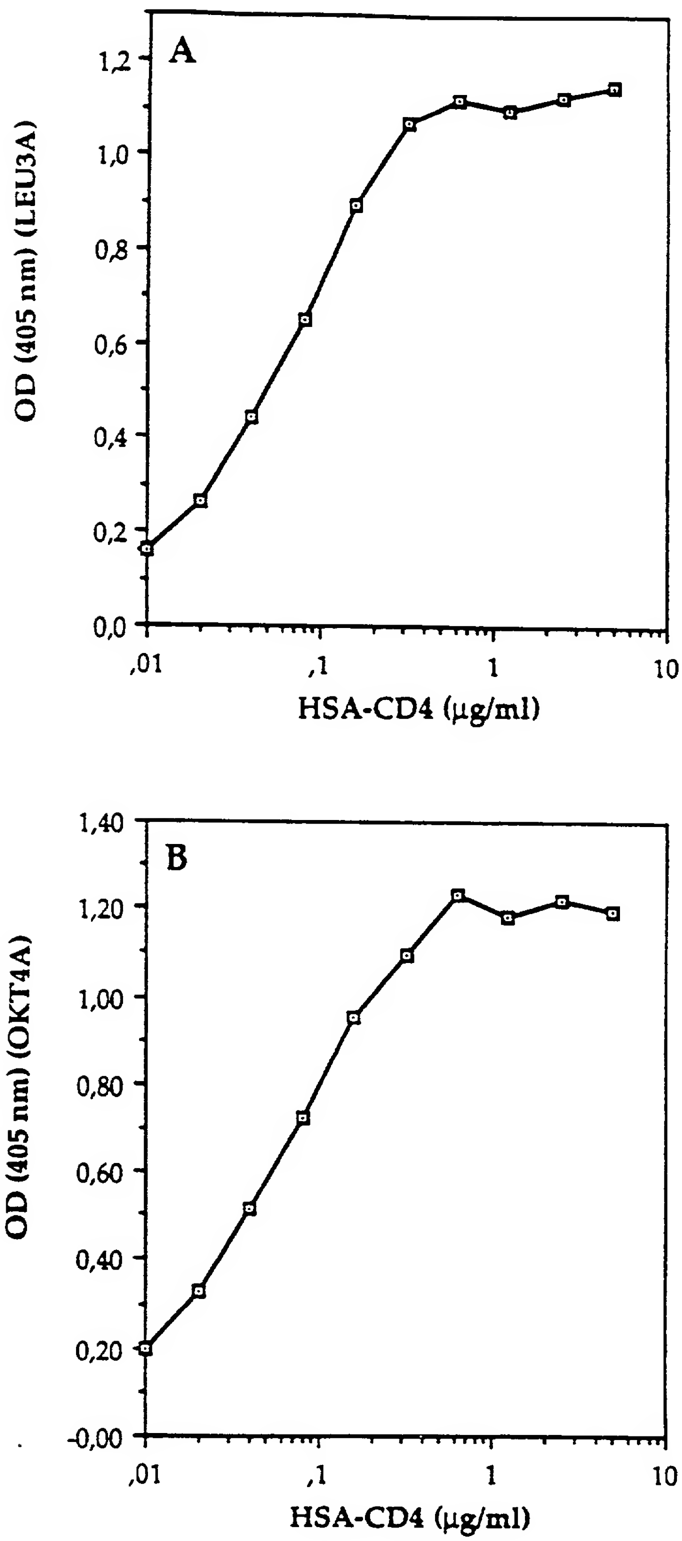

A
OD (405 nm) (LEU3A)
1,2
1,0
0,8
0,6
0,4
0,2
0,0
,01
,1
1
10
HSA-CD4 (µg/ml)
B
OD (405 nm) (OKT4A)
1,40
1,20
1,00
0,80
0,60
0,40
0,20
-0,00
,01
,1
1
10
HSA-CD4 (µg/ml)

Figure 20

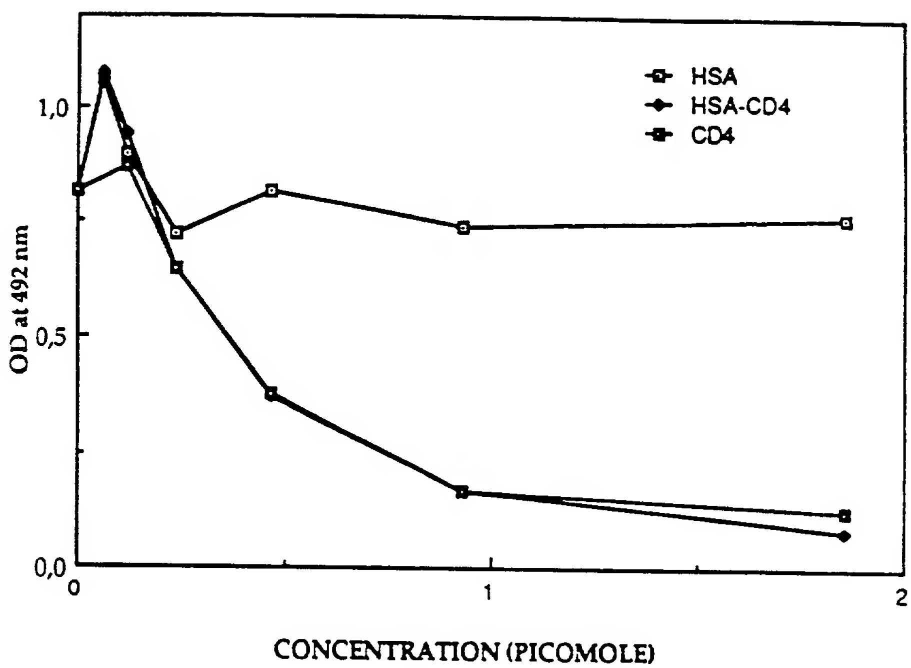

HSA
HSA-CD4
CD4
1,0
0,5
0,0
OD at 492 nm
0
1
2
CONCENTRATION (PICOMOLE)

Figure 21

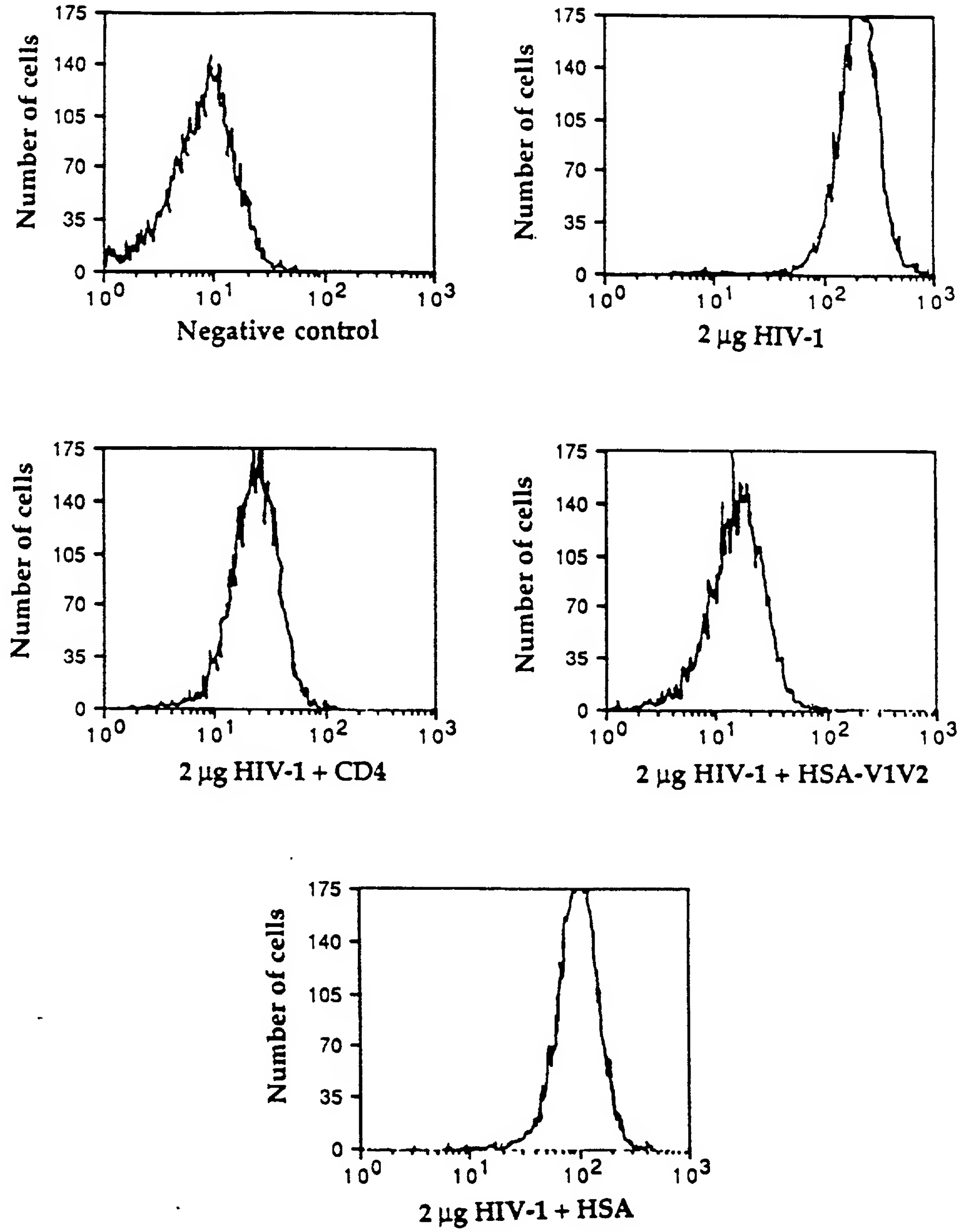

Number of cells
175
140
105
70
35
0
10^0
10^1
10^2
10^3
Negative control
2 μg HIV-1
2 μg HIV-1 + CD4
2 μg HIV-1 + HSA-V1V2
2 μg HIV-1 + HSA

Figure 22A

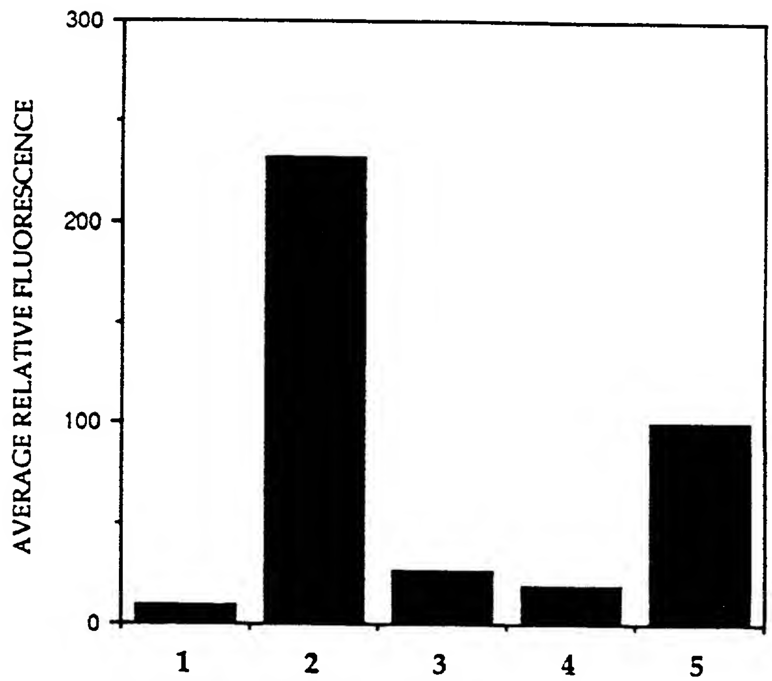
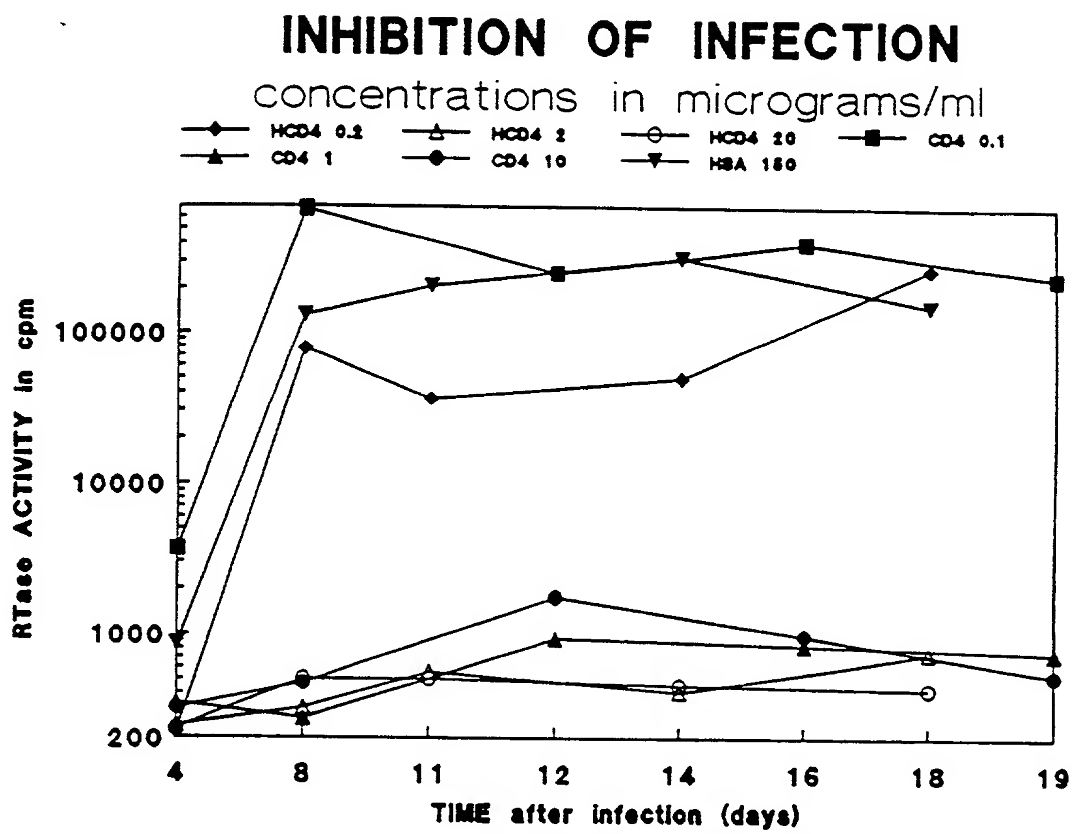


Figure 22B



20212020 " 02112001

Figure 23

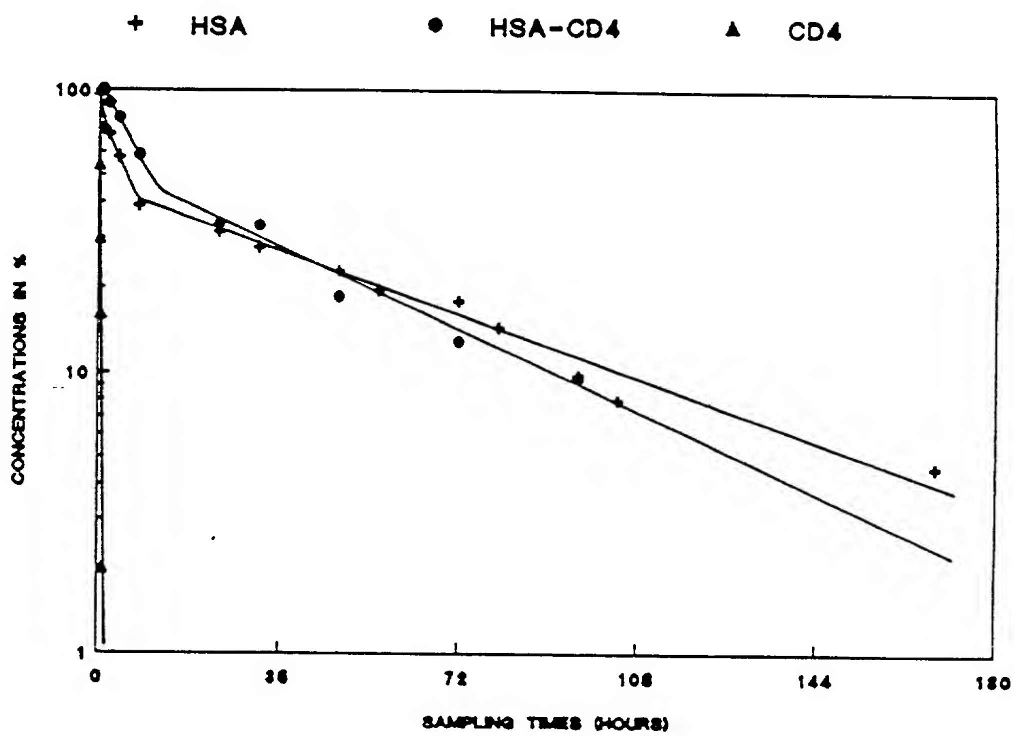


Figure 24

20212015 "C03E40T

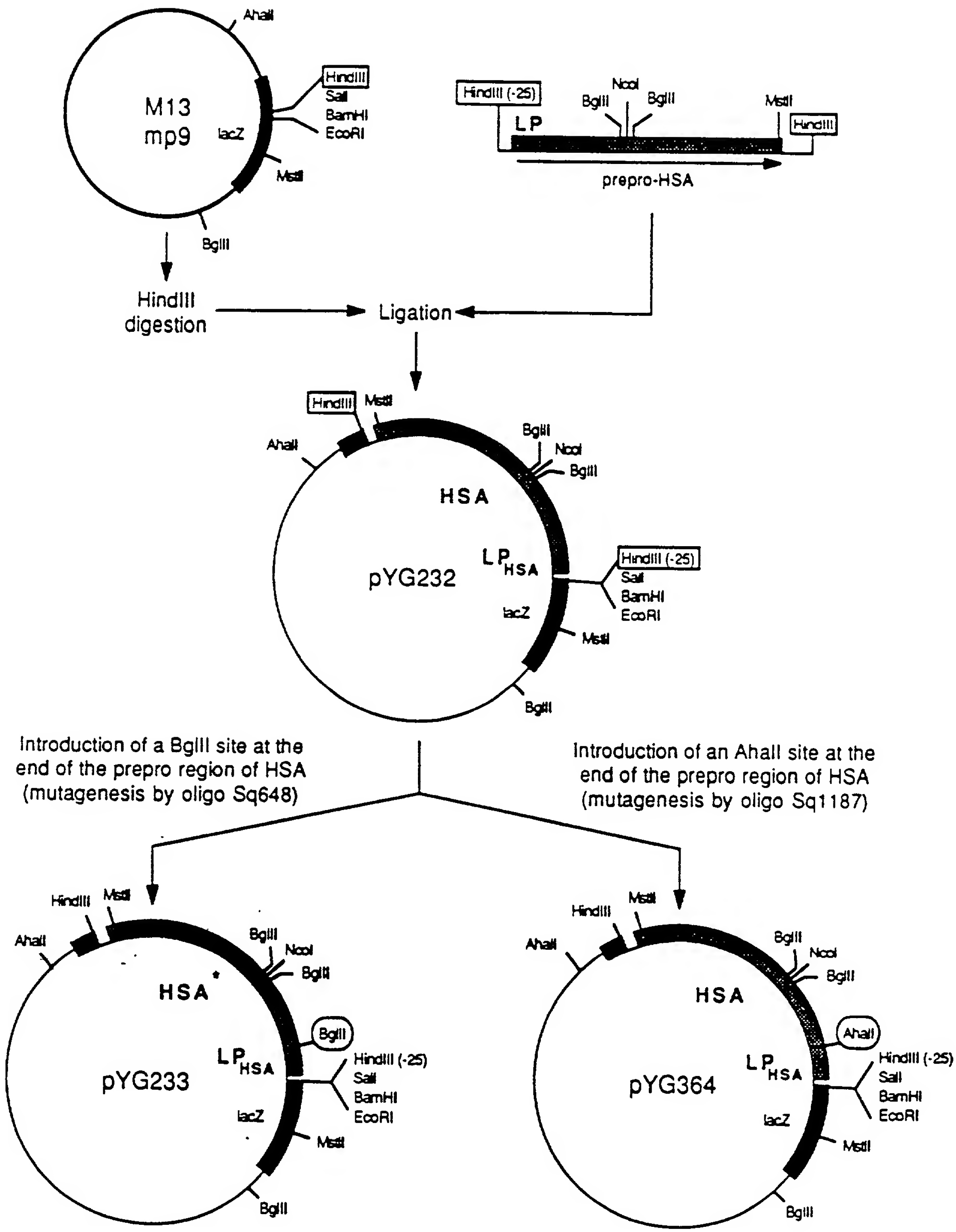

M13
mp9
lacZ
AhaII
HindIII
SalI
BamHI
EcoRI
MstII
BglII
HindIII (-25)
NcoI
LP
prepro-HSA
HindIII
digestion
Ligation
HSA
pYG232
LP_{HSA}
Introduction of a BglII site at the end of the prepro region of HSA (mutagenesis by oligo Sq648)
Introduction of an AhaII site at the end of the prepro region of HSA (mutagenesis by oligo Sq1187)
pYG233
HSA*
pYG364

Figure 25

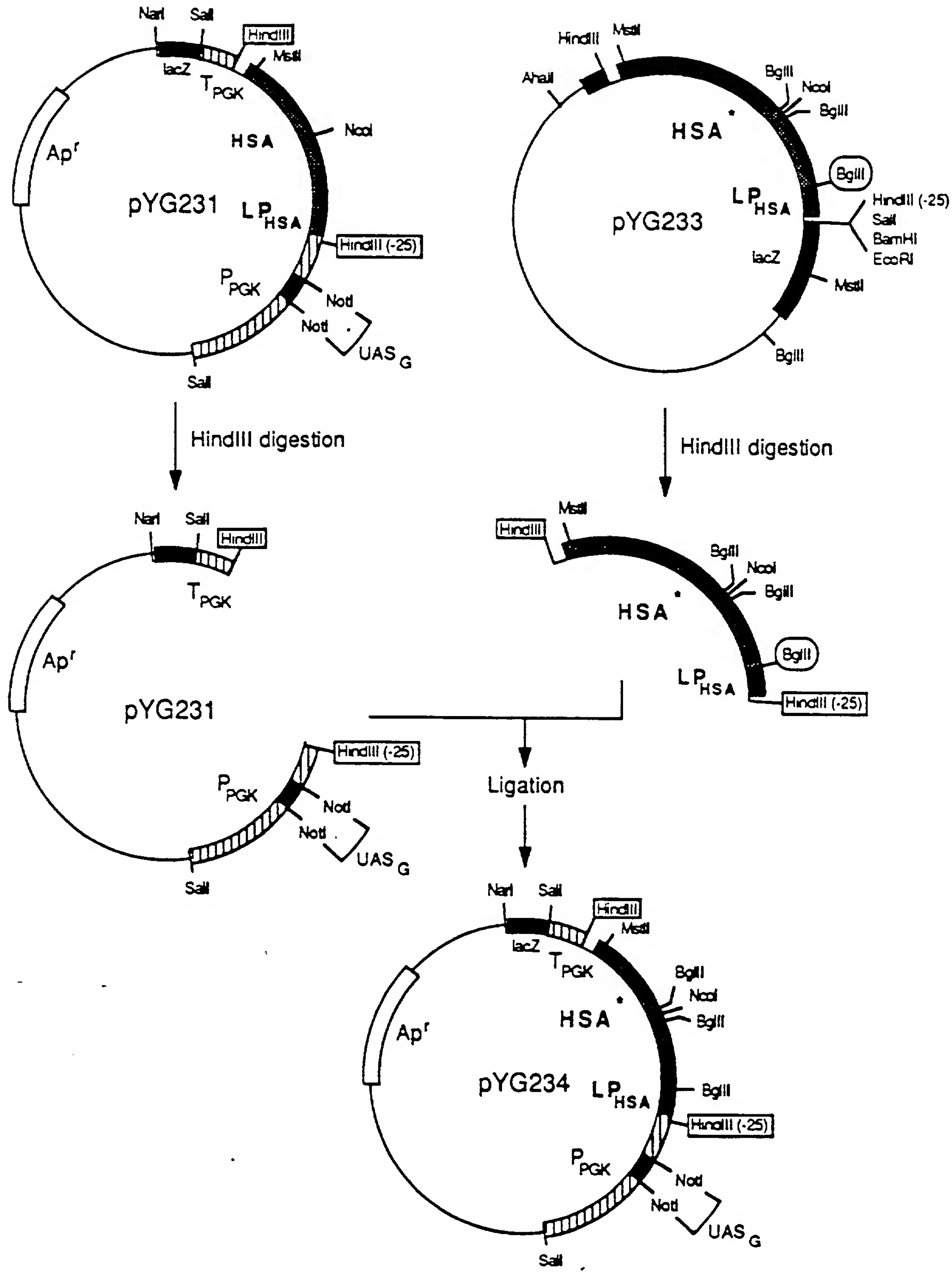

NarI
SalI
HindIII
MstII
lacZ
T_{PGK}
NcoI
HSA
Ap^r
pYG231
LP_{HSA}
HindIII (-25)
P_{PGK}
NotI
NotI
UAS_G
SalI
HindIII
MstII
AhaII
BglII
NcoI
BglII
HSA*
BglII
LP_{HSA}
HindIII (-25)
SalI
BamHI
EcoRI
pYG233
lacZ
MstII
BglII
HindIII digestion
HindIII digestion
Ligation
pYG234

Figure 26

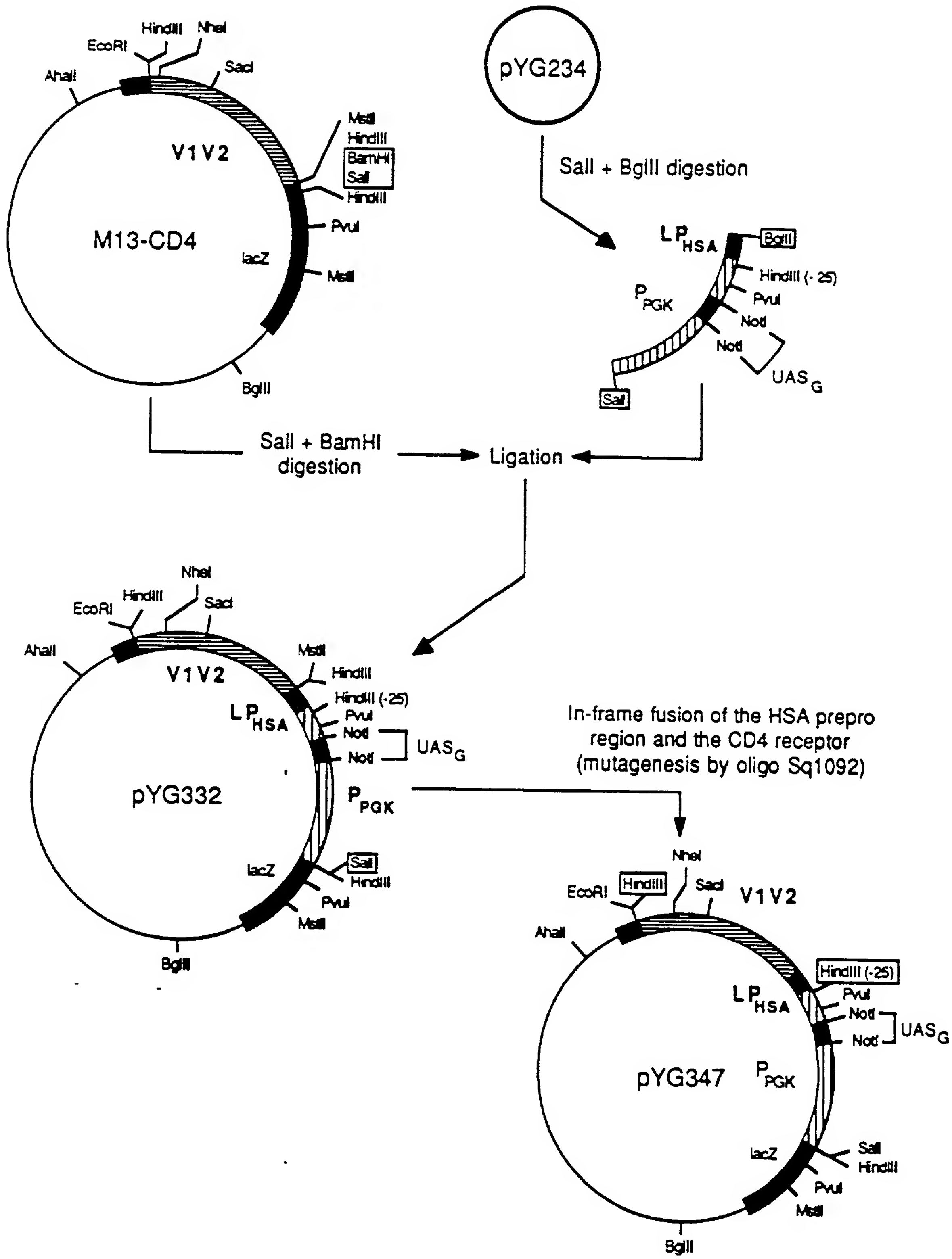


Figure 27

10072513.021202

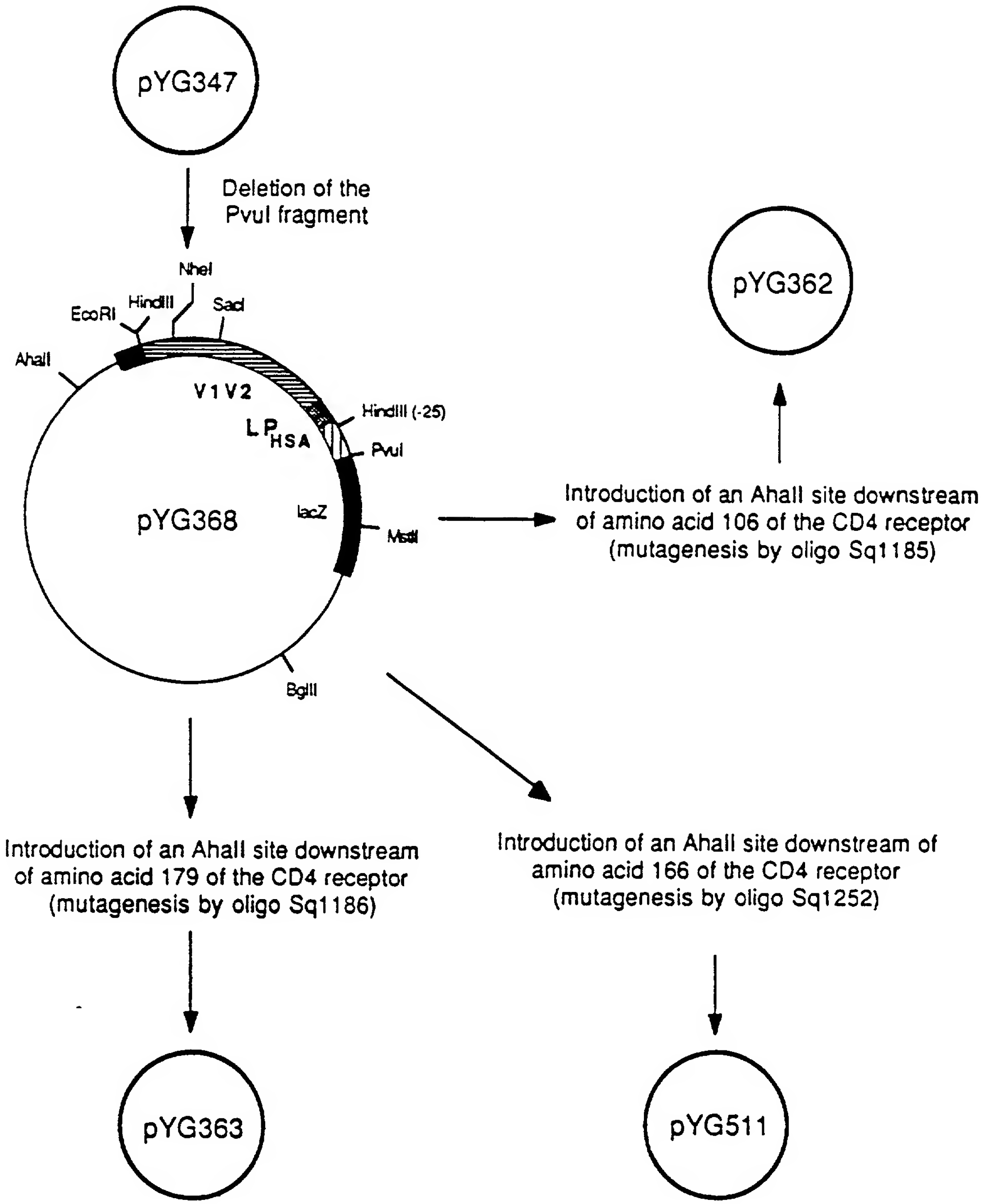

pYG347
Deletion of the
PvuI fragment
NheI
HindIII
SacI
EcoRI
AhaII
V1V2
LP HSA
HindIII (-25)
PvuI
pYG368
lacZ
MstII
BglII
pYG362
Introduction of an AhaII site downstream
of amino acid 106 of the CD4 receptor
(mutagenesis by oligo Sq1185)
Introduction of an AhaII site downstream
of amino acid 179 of the CD4 receptor
(mutagenesis by oligo Sq1186)
Introduction of an AhaII site downstream of
amino acid 166 of the CD4 receptor
(mutagenesis by oligo Sq1252)
pYG363
pYG511

Figure 28

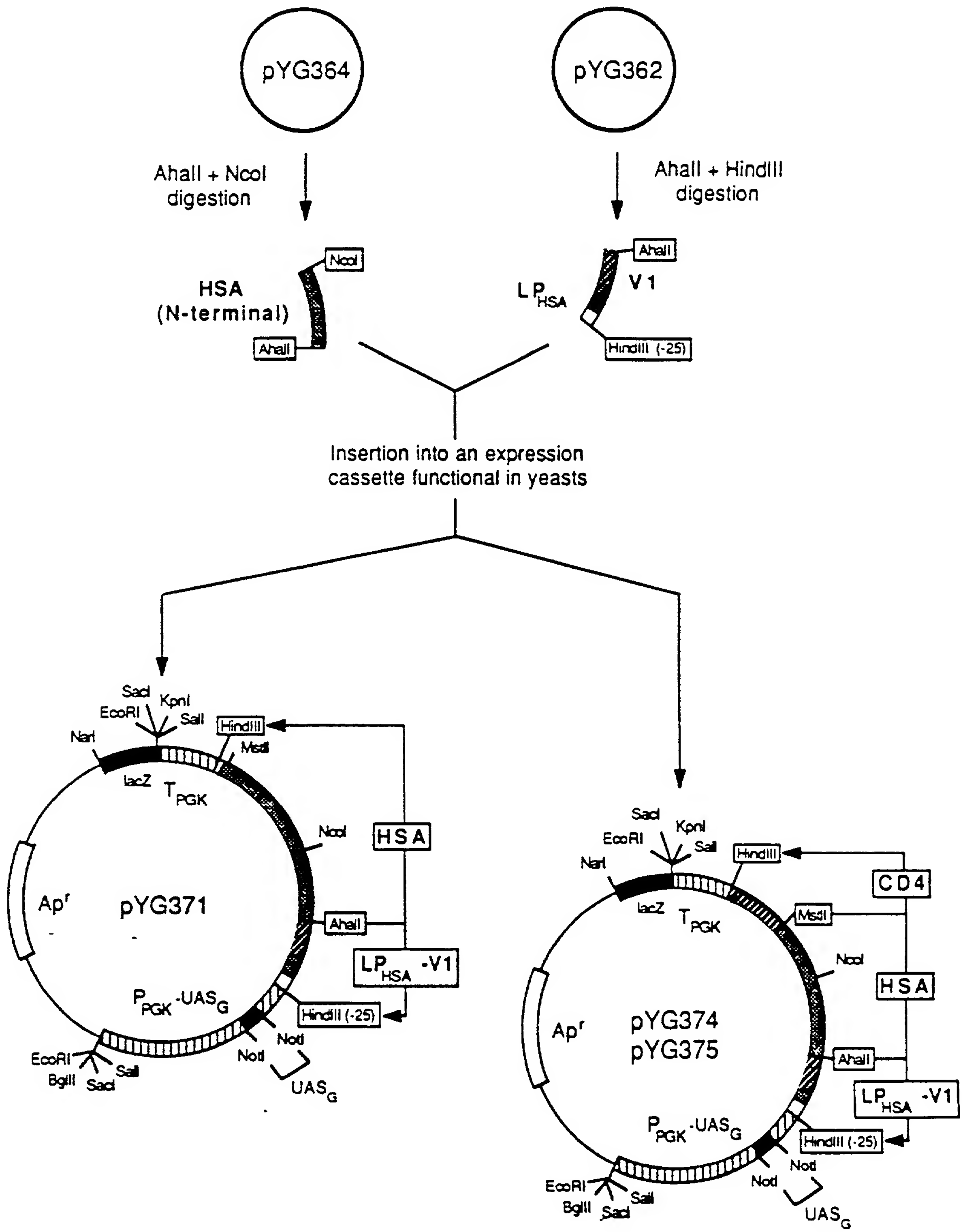


Figure 29

2021202.0314370 0

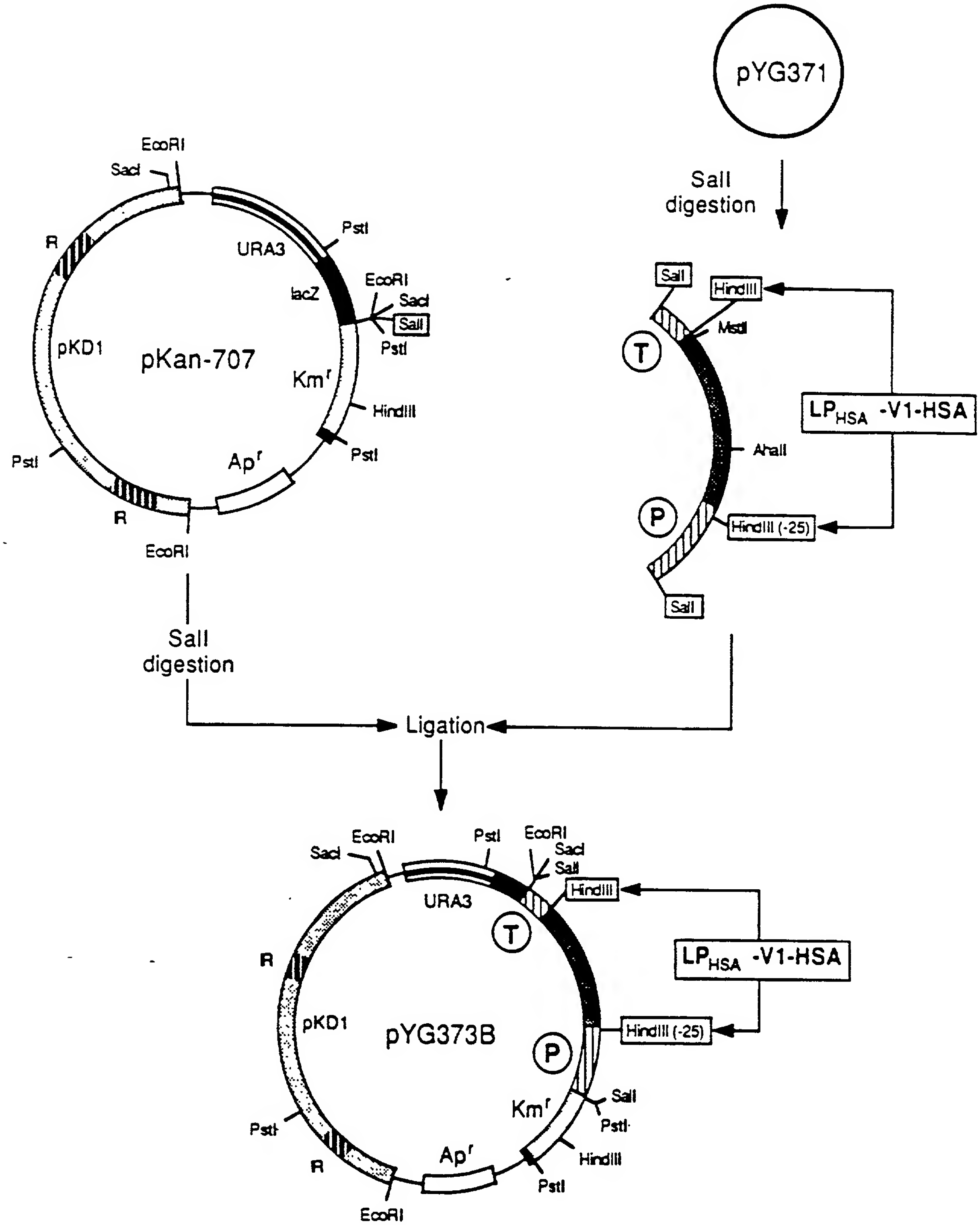

pYG371
SalI
digestion
EcoRI
SacI
R
URA3
PstI
lacZ
EcoRI
SacI
SalI
PstI
pKD1
pKan-707
Km^r
HindIII
PstI
Ap^r
PstI
R
EcoRI
SalI
HindIII
MstII
T
LP_HSA -V1-HSA
AhaII
P
HindIII (-25)
SalI
SalI
digestion
Ligation
SacI
EcoRI
PstI
EcoRI
SacI
SalI
HindIII
URA3
T
R
LP_HSA -V1-HSA
pKD1
pYG373B
HindIII (-25)
P
SalI
Km^r
PstI
PstI
HindIII
Ap^r
R
PstI
EcoRI

Figure 30

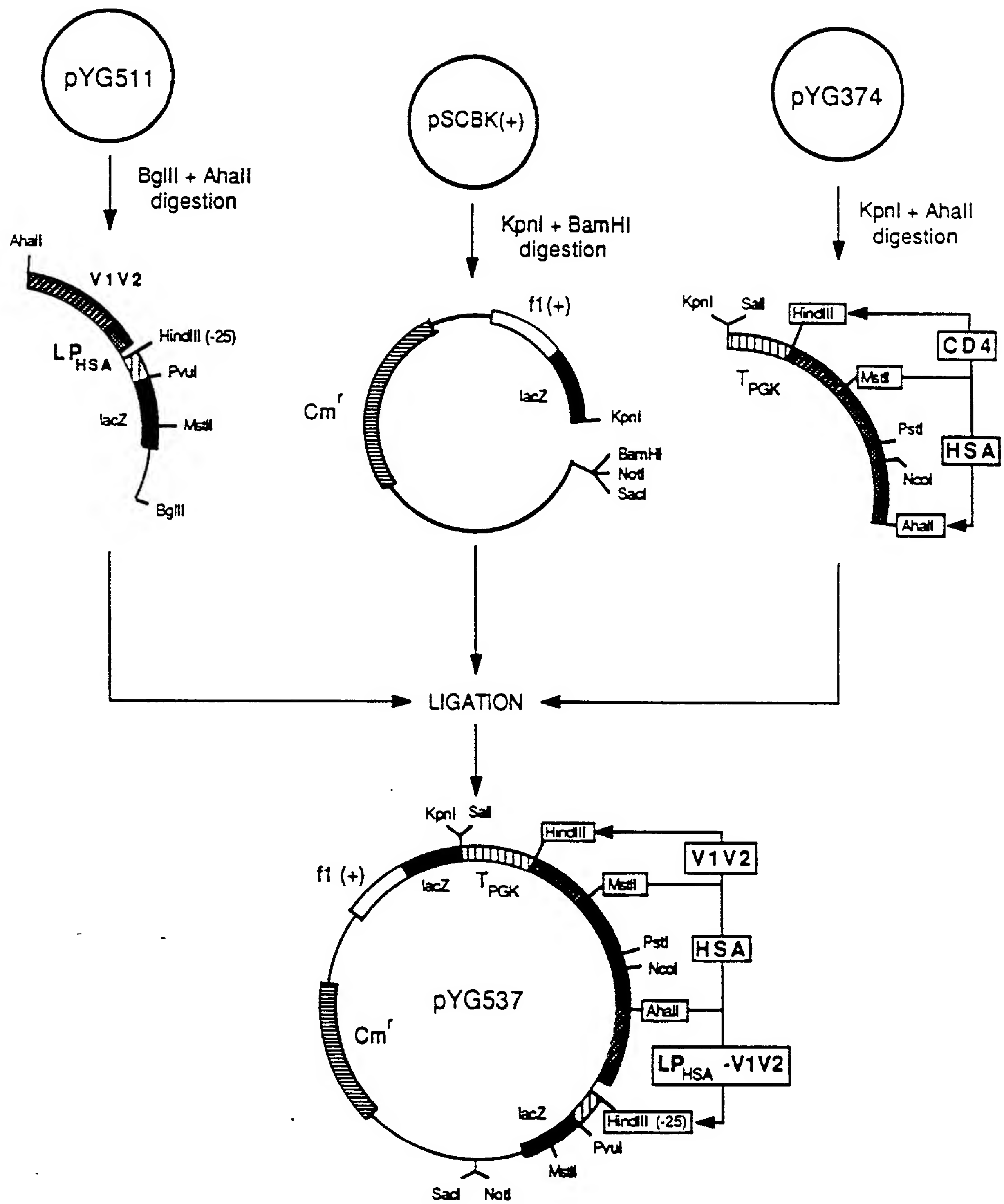


Figure 31

20520 "B1100B1"

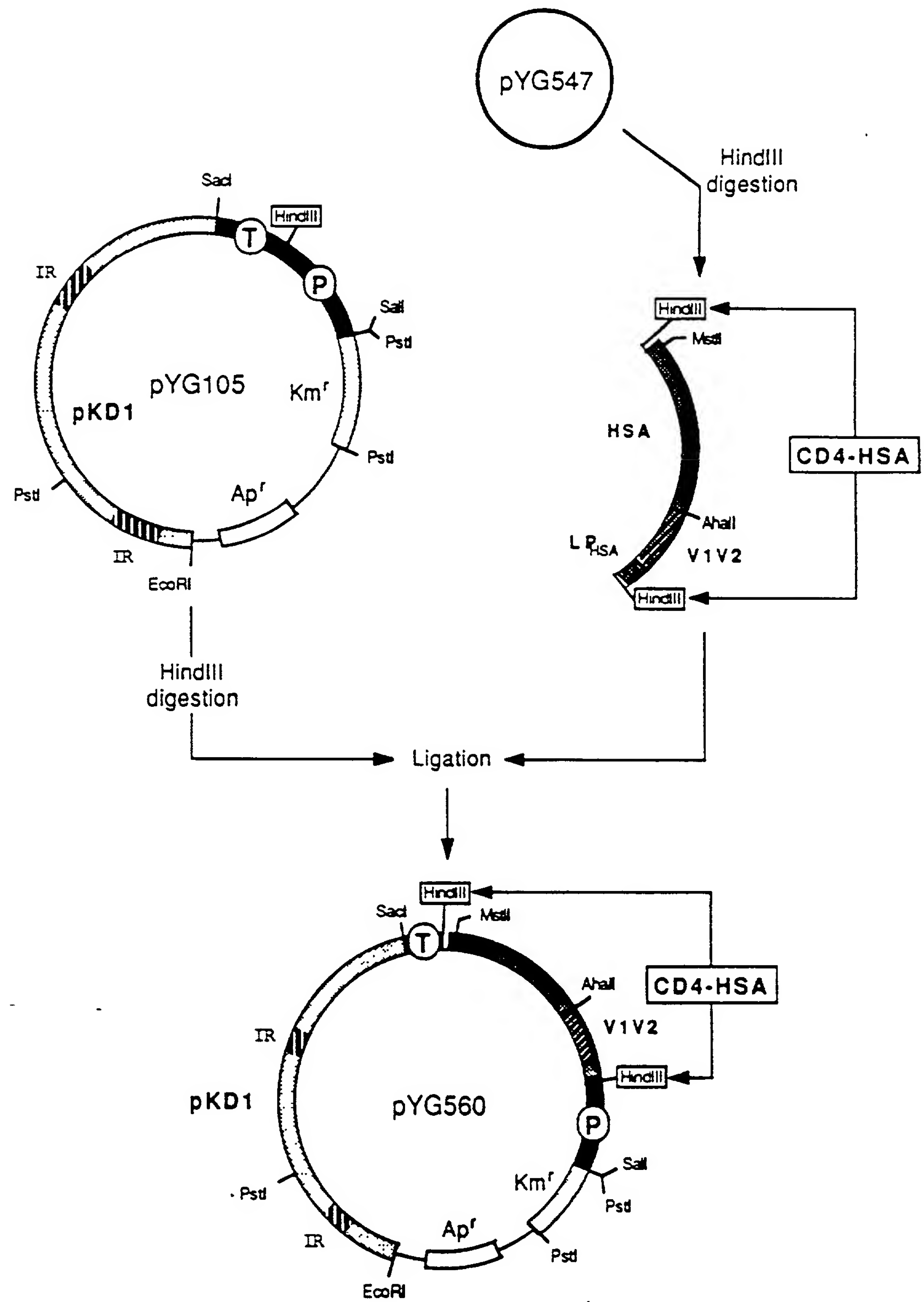


Figure 32

10073118 .021202

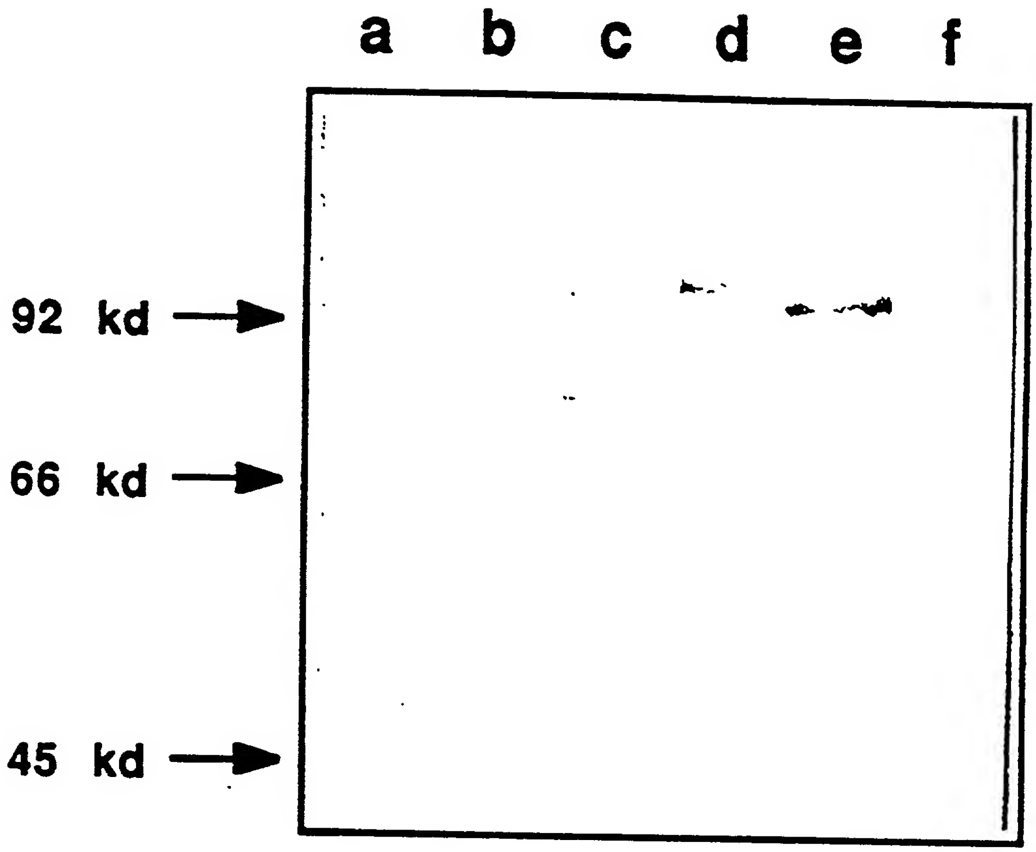


Figure 33

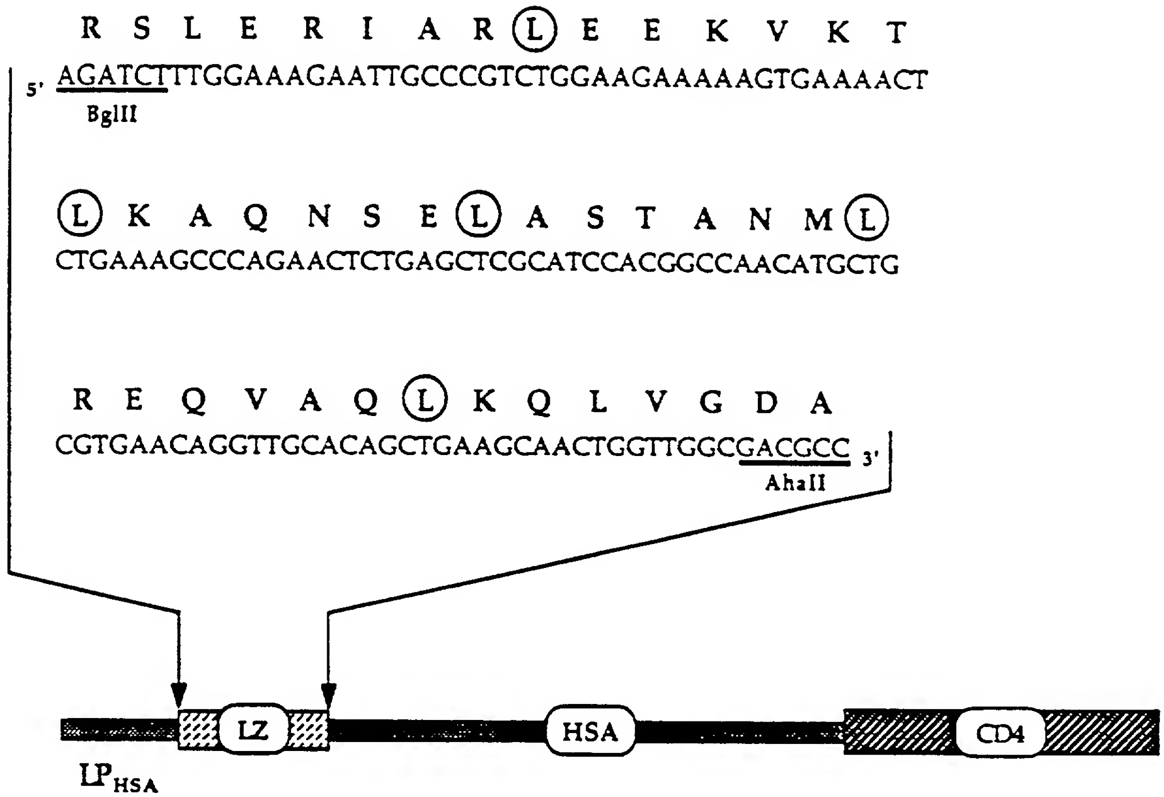

R S L E R I A R L E E K V K T
5' AGATCTTTGGAAAGAATTGCCCGTCTGGAAGAAAAAGTGAAAACT
BglII
L K A Q N S E L A S T A N M L
CTGAAAGCCCAGAACTCTGAGCTCGCATCCACGGCCAACATGCTG
R E Q V A Q L K Q L V G D A
CGTGAACAGGTTGCACAGCTGAAGCAACTGGTTGGCGACGCC 3'
AhaII
LZ
HSA
CD4
LP HSA

Figure 34

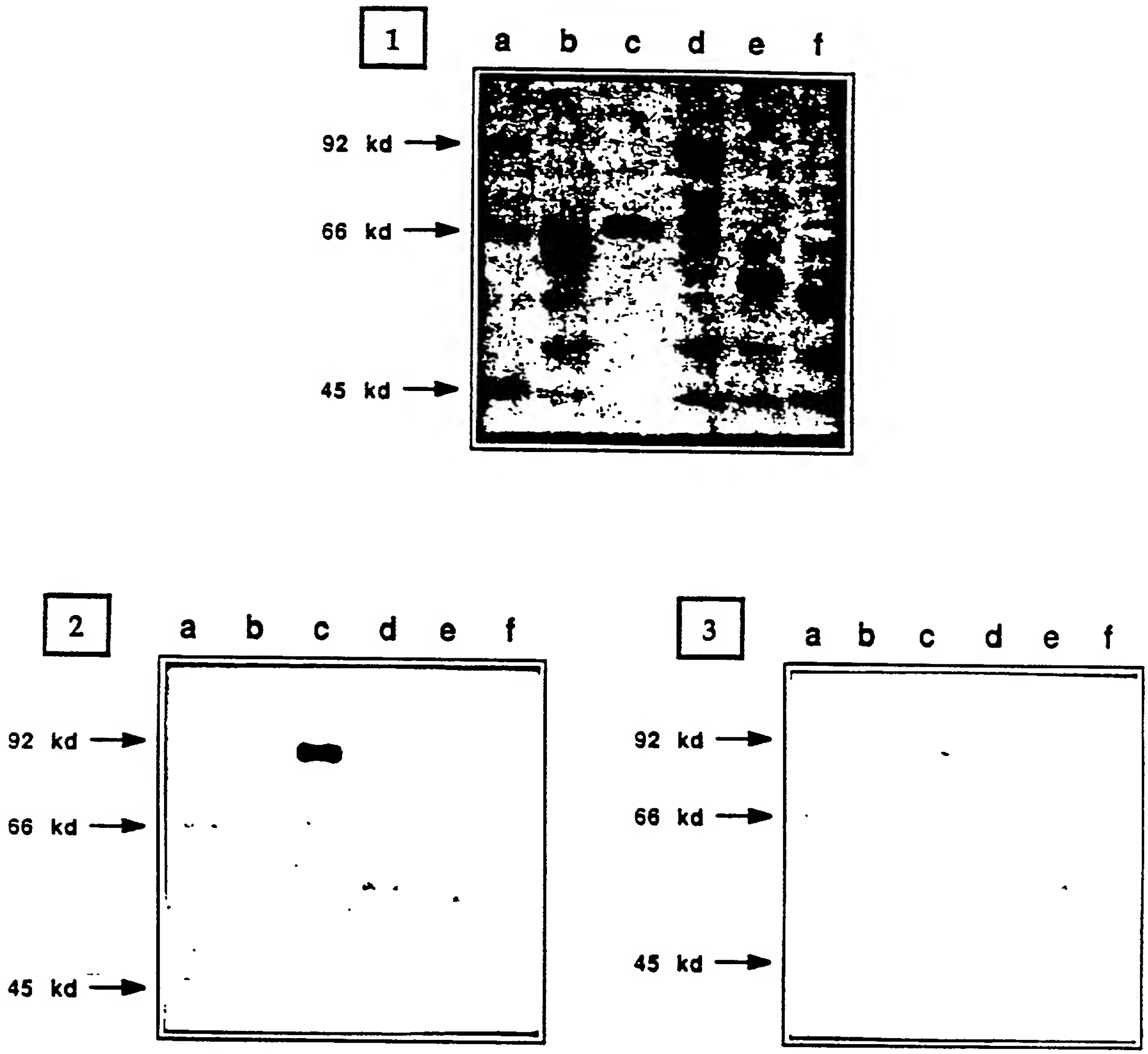

1
a b c d e f
92 kd
66 kd
45 kd
2
a b c d e f
92 kd
66 kd
45 kd
3
a b c d e f
92 kd
66 kd
45 kd

Figure 35

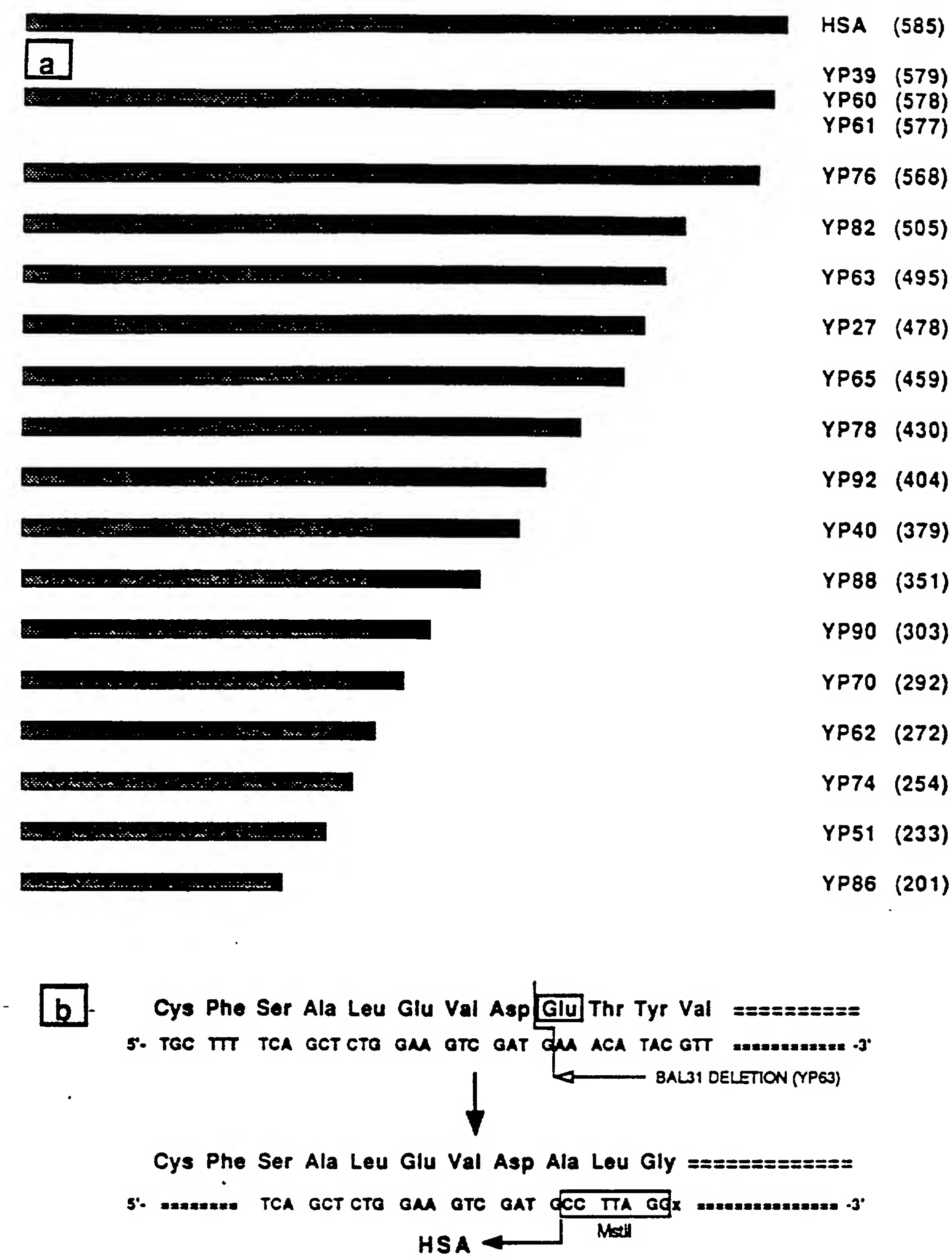

HSA (585)
a
YP39 (579)
YP60 (578)
YP61 (577)
YP76 (568)
YP82 (505)
YP63 (495)
YP27 (478)
YP65 (459)
YP78 (430)
YP92 (404)
YP40 (379)
YP88 (351)
YP90 (303)
YP70 (292)
YP62 (272)
YP74 (254)
YP51 (233)
YP86 (201)
b
Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val
5'- TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT -3'
BAL31 DELETION (YP63)
Cys Phe Ser Ala Leu Glu Val Asp Ala Leu Gly
5'- TCA GCT CTG GAA GTC GAT GCC TTA GGx -3'
MstII
HSA

Figure 36

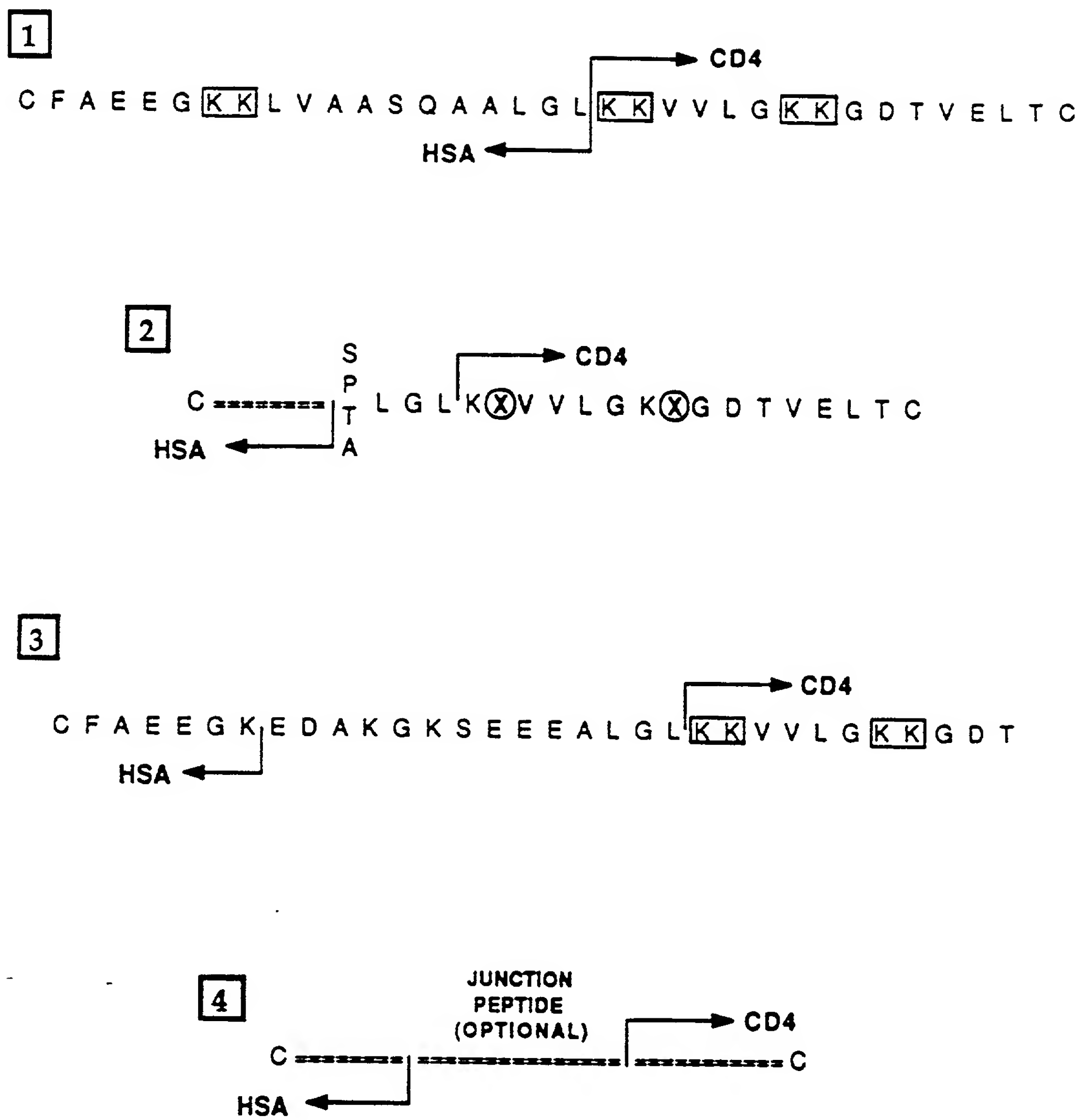

1
CD4
C F A E E G K K L V A A S Q A A L G L K K V V L G K K G D T V E L T C
HSA
2
CD4
C S P T A L G L K X V V L G K X G D T V E L T C
HSA
3
CD4
C F A E E G K E D A K G K S E E E A L G L K K V V L G K K G D T
HSA
4
JUNCTION PEPTIDE (OPTIONAL)
CD4
C C
HSA

Figure 37

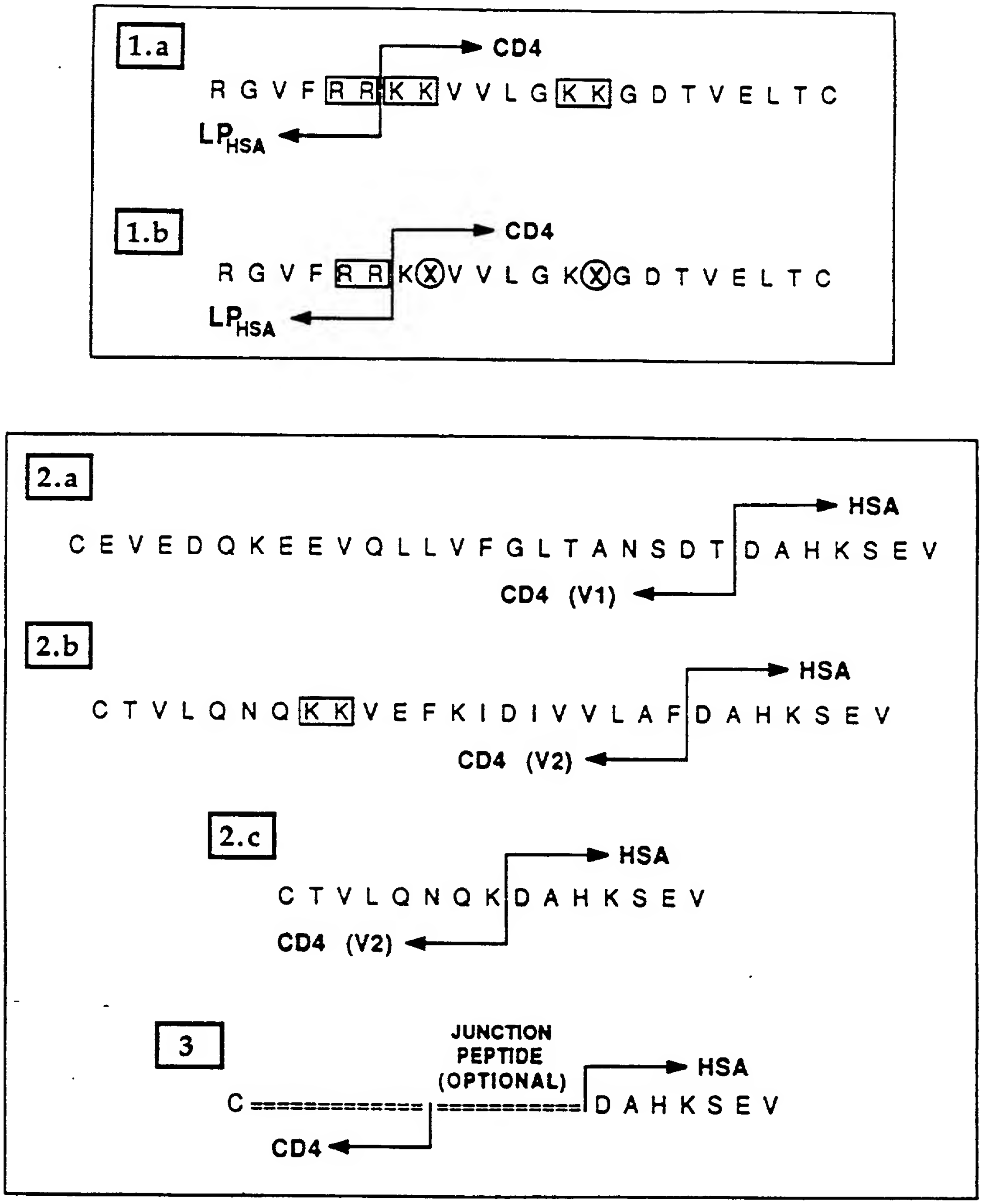

1.a
CD4
R G V F R R K K V V L G K K G D T V E L T C
LPHSA
1.b
CD4
R G V F R R K X V V L G K X G D T V E L T C
LPHSA
2.a
HSA
C E V E D Q K E E V Q L L V F G L T A N S D T D A H K S E V
CD4 (V1)
2.b
HSA
C T V L Q N Q K K V E F K I D I V V L A F D A H K S E V
CD4 (V2)
2.c
HSA
C T V L Q N Q K D A H K S E V
CD4 (V2)
3
JUNCTION
PEPTIDE
(OPTIONAL)
HSA
C
D A H K S E V
CD4

Figure 38